

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number  
**WO 01/17333 A1**

(51) International Patent Classification<sup>7</sup>: A01H 5/00, 5/10

(21) International Application Number: PCT/US00/24490

(22) International Filing Date:  
7 September 2000 (07.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
09/394,272 10 September 1999 (10.09.1999) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:  
— With international search report.

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 01/17333 A1

(54) Title: TRANSGENIC FIBER PRODUCING PLANTS WITH INCREASED EXPRESSION OF SUCROSE PHOSPHATE SYNTHASE

(57) Abstract: The present invention relates to a method of controlling the cellulose synthesis in plants to optimize the level of production and quality of the products derived from the plant. In particular, the present invention provides a transgenic cotton plant that has higher yields of cotton fiber and seed. The invention also provides methods for increasing the quality of cotton fiber produced from a cotton plant. The invention also provides general methods of changing the ratio of cellulose to other dry weight components of the plant, for changing the thickness of cell walls, for increasing the yield and changing the quality of other plant fibers, for increasing seed yield, and for increasing the tolerance of photosynthetic efficiency to cool night temperatures.

## **TRANSGENIC FIBER PRODUCING PLANTS WITH INCREASED EXPRESSION OF SUCROSE PHOSPHATE SYNTHASE**

### **FIELD OF THE INVENTION**

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The present invention relates to a method for increasing the yield or quality of product from a plant by altering the expression of sucrose phosphate synthase. In particular, the present invention provides a transgenic cotton plant that has an increased level of sucrose phosphate synthetase relative to a non-transgenic cotton plant. Methods  
10 are also provided for increasing the yield or the quality of cotton fiber and the yield of cotton seed produced from a cotton plant. General methods are provided for regulating the thickness of cell walls, for increasing the yield and quality of other plant fibers, for regulating the ratio of cellulose to other dry weight components of the plant, for increasing seed yield, and for increasing tolerance of photosynthetic efficiency to cool  
15 night temperatures.

### **BACKGROUND OF THE INVENTION**

The control of high-rate cellulose production and its regulation by temperature are  
20 critical to agriculture, since all plant growth (and hence the production of all food crops) depends on cellulose synthesis to build cell walls throughout the vegetative and reproductive parts of the plant. The cellulose within the primary walls of all cells of the plant body is also of direct industrial importance as a digestible part of animal forage and for manufacture of thickeners, ethanol, and other cellulose-based or cellulose-derived  
25 products. Furthermore, plant parts based on secondary cell walls with high cellulose content are contained in or compose economically important plant products, including cotton fibers, wood, and fibers in forage crops. The agronomic productivity and product quality of wood and cotton, as well as other fiber crops such as hemp and flax, are in large part determined by the biosynthesis of cellulose. Therefore, an understanding of the  
30 basic regulatory mechanisms of cellulose synthesis and how it responds to temperature stress allows for beneficial changes in crop plants (improved product yield and quality) through genetic engineering.

Since cotton fiber weight is more than 90% cellulose, cotton is one particular crop where enhancing the flow of carbon to cellulose production can increase yield and

quality. This will be an especially beneficial outcome if it is achievable under diverse environmental conditions encountered in cotton production fields, including cool night temperatures that hinder cotton fiber development. For example, it is known that cool night temperatures hinder the seasonal yield and quality of cotton fiber (Gipson,  
5 "Temperature Effects on Growth, Development, and Fiber Properties," in Mauney, eds., Cotton Physiology, The Cotton Foundation:Memphis, pp. 47-56) because they hinder the rate of cellulose synthesis (Roberts et al., "Effects of Cycling Temperatures on Fiber Metabolism in Cultured Cotton Ovules," Plant Physiol., 100:979-986 (1992)). The ability to manipulate cotton yield and fiber quality parameters and sustain or improve them  
10 under diverse and/or stressful environmental conditions will allow for beneficial changes in crop plants (improved product quality) through genetic engineering.

Cotton fiber yield is the most important determinant of the value of the crop to the producer. Reputable cotton breeders have recently pointed out that cotton production has reached a fiber yield plateau, which bodes ill for the financial success of producers given  
15 escalating costs. Potential contributors to this problem include the environmental sensitivity of cotton fiber and seed development, the narrow genetic base of commercial cotton, and the recent introduction of transgenic traits such as herbicide and insect resistance through back-crossing with transformed *Gossypium hirsutum* cv. Coker 312. Coker 312 (C312) is an old cultivar frequently used for transformation because of its high  
20 regeneration capacity. Use of genetic engineering to make cotton crop production more stress resistant, to expand the genetic potential of cultivated cotton, and to improve the yield of transformed cotton with diverse novel traits will bring needed increases in crop yield.

Similarly, seed yield is of value to the cotton producer since seeds are sold for oil  
25 production and animal feed. Another minor component, the short fuzz fibers on each seed, provides added economic value to the seed crop. Increased seed and fuzz fiber yield without sacrifice of lint fiber yield or quality would help the producer recover more profit per acre of cotton production. As for cotton seed, increased yield of any seed crop will be of major benefit to agriculture.

30 Improved cotton fiber quality parameters such as micronaire, maturity ratio, length, length uniformity, bundle strength, and single fiber strength are desired by the textile industry to produce increasingly high quality products and to take full advantage of modern spinning technologies. Fiber quality parameters should also be high enough for

the cotton producer to avoid price discounts when he sells his crop to the gin. For example, in a short growing season on the Texas Southern High Plains, producers often suffer price discounts due to low micronaire. Increasingly high fiber quality achieved through breeding has become a required standard in the cotton industry, and market  
5 forces may change so producers are more routinely rewarded with price premiums for higher quality cotton. Therefore, stabilizing or increasing fiber quality under diverse environmental conditions through genetic engineering will increase the profitability of cotton crop production and provide a new spectrum of material properties for exploitation by the processing industries.

10 Other plant fibers, although often of different tissue origin, share structural features in common with cotton fibers in being elongated cells with cellulose-rich walls. Like cotton fibers, other plant fibers of industrial use are required to have high quality as defined by factors such as cellulose content and wall thickness, diameter, fineness (or coarseness), length, strength, durability, uniformity, elasticity, and elongation. There is  
15 an optimum range of such parameters for each particular fiber source and industrial use. Taking examples from wood fibers used after pulping in paper production, longer fiber length and higher single fiber elongation both promote higher paper tear strength. In addition, thick fiber walls promote high pulp yield and production of absorbent paper with high tearing resistance. However, thinner fiber walls promote fiber collapse and  
20 better inter-fiber bonding that aids production of high quality writing paper. Therefore, there exists a need to control cell wall thickness and other fiber quality parameters in either negative or positive directions in diverse fibers to improve their yield or quality or expand the range of their industrial utility.

Maximizing crop productivity and utility per acre is a key component of  
25 sustainable agriculture. Enhanced production of multiple products from the same crop, such as seed and fiber, would be useful. Similarly, it will be an advantage to maximize the possibility of a successful crop harvest, for example by generating plants with stiffer stems that can better resist lodging in the field without sacrificing the yield of a seed crop.

An increasing level of CO<sub>2</sub> in the atmosphere is a concern due to predicted  
30 association of rising global temperatures. There exists a need for plants that are better able to immobilize CO<sub>2</sub> by conversion of it into useful products, especially products that are typically not burned to regenerate CO<sub>2</sub>.



Cotton leaves assimilate most carbon into starch during the day, and the starch is converted to sucrose at night for translocation to sinks. As just described, cotton fibers are not well adapted to use this sucrose efficiently for cellulose synthesis during cool nights. Therefore, cool nights reduce cotton photosynthetic efficiency during the  
5 following warm day (Warner et al., "Response of Carbon Metabolism to Night Temperatures in Cotton," Agron. J., 87:1193-1197 (1995)), possibly due to hindered use of carbohydrate at night. The resulting leaf carbohydrate accumulation could signal a down-regulation of photosynthetic genes. The excess starch remaining in the leaf after a cool night could be involved in some negative feedback mechanism reducing  
10 photosynthetic rates even after re-warming. There is a need to use genetic engineering to alleviate the cool-night-associated inhibition of photosynthesis during the following warm day.

Sucrose phosphate synthase ("SPS") is a key protein involved in carbon metabolism in plants (See Figure 1). SPS catalyzes the formation of sucrose phosphate  
15 from UDP-glucose and fructose 6-phosphate. In the leaf, SPS is important in controlling the partitioning of reduced carbon between starch and translocatable sucrose (Huber et al., "Role and Regulation of Sucrose-Phosphate Synthase in Higher Plants," Annu. Rev. Plant Physiol. Plant Mol. Biol., 47:431-44 (1996)). In growing sink cells, the data in this invention demonstrate that SPS is involved in directing the flow of carbon to cellulose.  
20 Its level of activity can regulate the amount of metabolic flux directed toward cellulose synthesis compared to respiration (See Figure 2). According to this model, SPS within cellulose-storing sink cells can increase sink strength through an enhanced rate of cellulose synthesis by promoting sucrose synthesis in one or both of two cases: (a) if sucrose transported from the leaves is cleaved to release glucose and fructose before or  
25 after entering the sink cells; and/or (b) to reuse the fructose released by the activity of sucrose synthase to channel UDP-glucose and fructose to cellulose synthase. A decreased level of SPS activity can decrease sink strength, by analogous mechanisms, in any case where sink filling is affected by sucrose levels.

In tomato, over-expression of SPS has been shown sometimes to cause a 32%  
30 increase in total fruit dry weight. This increase was due not to an increase in individual fruit weight, but to a 50% increase in fruit number (Micallef et al., "Altered Photosynthesis, Flowering, and Fruiting in Transgenic Tomato Plants That Have an Increased Capacity for Sucrose Synthesis," Planta, 196:327-334 (1995)). These tomato

plants have also sometimes been shown to have increased fresh fruit weight per fruit and increased fruit soluble solids (sugars) (Laporte et al., "Sucrose-Phosphate Synthase Activity and Yield Analysis of Tomato Plants Transformed with Maize Sucrose-Phosphate Synthase," Planta, 203:253-259 (1997)). These reports provide no information  
5 about seed yield since tomato seeds weigh little compared to tomato fruits and seeds were not separated from fruits for weighing.

It should be noted that although cotton bolls and tomatoes are both classified botanically as fruits, the nature of the fruits and the relative importance of the seeds they contain is very different. Tomato fruits are essentially sacks of primary cell walls filled  
10 with water and soluble glucose, fructose, and sucrose as storage carbohydrates. These sugars crystallize upon drying, contributing to fruit dry weight. Within the fruit, tomato seeds are not a significant sink due to their small size, and they have no economic value except for propagation of tomato. The fruit is the major sink in tomatoes; it constitutes almost all of tomato yield and is the only tomato part with significant economic value.

15 In contrast, the cotton fruit is relatively dry and thin-walled. The fruit itself does not constitute any substantial sink in cotton or contribute to cotton yield. It protects the seeds only until boll opening, after which it withers. The fruit has no or little economic value (as compost). Cotton seeds with attached fiber represent the two major sinks of substantial economic value in the cotton crop. The cotton fiber is an elongated epidermal  
20 cell of the cotton seed coat; it is defined botanically as a trichome. Therefore, the two major sinks in seeds are: (1) the cotyledons of the seed embryo that store oil and protein; and (2) the secondary cell walls of the seed epidermal trichomes (cotton fibers) that store insoluble cellulose. Soluble sugars are not stored in any significant quantity in a mature cotton seed or fruit. Cotton seeds with their attached fiber represent all of the yield in the  
25 cotton crop. Therefore, cotton, as well as other fiber producing plants, differ significantly from tomato.

Increased total dry weight of vegetative parts of plants over-expressing SPS has been shown in tomato leaves. In the same study, no change was observed in dry weight of stems and root dry weight decreased (Galtier et al., "Effects of Elevated Sucrose-  
30 Phosphate Synthase Activity on Photosynthesis, Assimilate Partitioning, and Growth in Tomato (*Lycopersicon esculentum* var UC82B)," Plant Physiol., 101:535-543 (1993)). Tomato leaves do not contain substantial fiber, being composed mainly of mesophyll cells and conducting vascular tissue. The same plants were shown to sometimes have

- increased dry weight on a whole-plant basis (Ferrario-Méry et al., "Manipulation of the Pathways of Sucrose Biosynthesis and Nitrogen Assimilation in Transformed Plants to Improve Photosynthesis and Productivity," in Foyer, eds., A Molecular Approach to Primary Metabolism in Higher Plants, Taylor and Francis:New York, pp. 125-153 (1997))
- 5 and in above-ground parts including leaves plus stems (Laporte et al., "Sucrose-Phosphate Synthase Activity and Yield Analysis of Tomato Plants Transformed with Maize Sucrose-Phosphate Synthase," Planta, 203:253-259 (1997)). In potatoes over-expressing SPS, increased total dry weight of tubers has been shown (Shewmaker, "Modification of Soluble Solids Using Sucrose Phosphate Synthase Encoding
- 10 Sequences," PCT International Publication Number WO 97/15678). Potato tubers do not contain substantial fiber. They are composed mainly of parenchyma cells with primary walls that store abundant starch and lesser amounts of protein. The major yield component of potato tubers is starch. All of these reports lack information on the effect of SPS over-expression on cell wall thickness, cellulose content, and fiber and seed yield
- 15 of plants. However, the absence of demonstrated increase in stem weight argues against increased fiber content in the tomato plants analyzed.

- Increased expression of SPS has been shown to exert other beneficial effects in tomato and *Arabidopsis*. In both species, leaf starch storage is reduced in preference for synthesis of sucrose. In both species, maximal rates of photosynthesis are enhanced, most
- 20 significantly in elevated CO<sub>2</sub> and saturating light (Galtier et al., "Effects of Light and Atmospheric Carbon Dioxide Enrichment on Photosynthesis and Carbon Partitioning in the Leaves of Tomato (*Lycopersicon esculentum* L.) Plant Over-Expressing Sucrose Phosphate Synthase," J. Expt. Bot., 46:1335-1344 (1995); Micallef et al., "Altered Photosynthesis, Flowering, and Fruiting in Transgenic Tomato Plants That Have an
- 25 Increased Capacity for Sucrose Synthesis," Planta, 196:327-334 (1995); and Signora et al., "Over-Expression of Sucrose Phosphate Synthase in *Arabidopsis thaliana* Results in Increased Foliar Carbohydrate Accumulation in Plants After Prolonged Growth with CO<sub>2</sub> Enrichment," J. Expt. Bot., 49:669-680 (1998)). However, these reports provide no information related to effects of cool nights on photosynthesis during the warm day.
- 30 Thus, there exists a need for a method to control the level of synthesis of cellulose in fiber producing plants, in particular cotton. There exists a need to be able to control the yield and quality of fibers of commercial value, in particular cotton, under diverse environmental conditions. A general need exists to be able to control the synthesis of

cellulose and the thickness of cell walls in plants. A general need exists to promote photosynthetic efficiency in plants growing under cool night temperatures. It is important to be able to increase seed yield in crops as well. The present invention addresses those needs and provides improved plants.

5

## SUMMARY OF THE INVENTION

The present invention generally relates to a method of controlling the cellulose synthesis in plants to optimize the level of production and quality of the products derived from the plants.

The invention includes the regulation in the cellulose content, thickness, or yield of any plant cell wall of agricultural or industrial use. Such cell walls include typical thin primary cell walls such as those that are digested in forage and those that exist in useful agricultural residues, for example beet root parenchyma cells remaining after sugar extraction that can be converted into thickening agents. Such cell walls include thick walls such as those of collenchyma and xylem parenchyma that can aid plant rigidity or contribute to yield and digestibility of forage or other agricultural products. Such cell walls also include secondary cell walls such as are commonly found in fiber.

In particular, the present invention provides a transgenic cotton plant that has an increased level of sucrose phosphate synthetase relative to a non-transgenic cotton plant.

The invention also provides a method of increasing the yield of a cotton plant by introducing into the cotton plant a chimeric DNA construct that alters the level of sucrose phosphate synthase activity in an amount sufficient to increase the seed and fiber yield of the cotton plant.

The present invention can also be used to increase the quality of cotton fiber produced from a cotton plant by introducing into a cotton plant a chimeric DNA construct that alters the level of sucrose phosphate synthase activity in an amount sufficient to increase the quality of the cotton fiber produced by the cotton plant.

The invention includes a method of increasing tolerance of photosynthetic efficiency to cool night temperatures by introducing into a plant a chimeric DNA that alters the sucrose phosphate synthase activity in an amount sufficient to increase tolerance of photosynthetic efficiency to cool night temperatures.

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In yet another embodiment, the invention provides a method of regulating the ratio of cellulose to other dry weight components of the plant by introducing into a plant a chimeric DNA construct capable of altering sucrose phosphate synthase activity in an amount sufficient to regulate the ratio of cellulose to other dry weight components of the plant.

The invention also provides a method of regulating the thickness of cell walls in a plant by introducing into a plant a chimeric DNA construct that alters sucrose phosphate synthase activity in an amount sufficient to regulate the thickness of cell walls.

In yet another embodiment, the invention provides a method of increasing the harvestable yield of fiber from a fiber containing plant by introducing into a plant a chimeric DNA construct that alters sucrose phosphate synthase activity in an amount sufficient to increase the harvestable yield of fiber from a fiber producing plant.

In yet another embodiment, the invention provides a method of increasing the harvestable yield of seed from a seed producing plant by introducing into a plant a chimeric DNA construct that alters sucrose phosphate synthase activity in an amount sufficient to increase the harvestable yield of seed from a seed producing plant.

In yet another embodiment, the invention provides a method of improving the quality of fiber from a fiber producing plant by introducing into a plant a chimeric DNA construct that alters sucrose phosphate synthase activity in an amount sufficient to regulate fiber quality. Such improvement may be exemplified by changes in length, strength, and weight per unit length.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the pathways of carbon assimilation, starch synthesis and catabolism, and sucrose synthesis. UDP-glucose pyrophosphorylase catalyzes the highly reversible reaction between glucose 1-phosphate (G-1-P) and UDP-glucose. Sucrose-phosphate synthase catalyzes the formation of sucrose-phosphate from UDP-glucose and fructose 6-phosphate.

Figure 2 shows the metabolic pathways and enzymes in sink cells related to the biosynthesis of cellulose.

Figure 3 is an amino acid alignment between SPS gene sequences from a number of plant species.

Figure 4 is an amino acid alignment between the spinach leaf SPS gene sequence and a homologous sequence from *Synechocystis*.

Figure 5 is a histogram of fiber weight per seed, which shows elevation in all three transgenic lines. (Here and in all subsequent histograms, the error bars are standard  
5 deviations of the average. The average values are printed above each bar.)

Figure 6 is a histogram of delinted seed weight per seed. It shows elevation in all three transgenic lines.

Figure 7 is a histogram of the ratio of fiber weight per seed and delinted seed weight per seed. It shows that these two yield parameters tend to increase in parallel,  
10 with a small preference for increased fiber weight in transgenic lines.

Figure 8 is a scatter plot of fiber weight per seed vs delinted seed weight per seed. It shows that these two parameters are interdependent at the 50% level. (Here and with all other scatter plots,  $R^2$  is the coefficient of determination calculated from the linear regression line. Also, data points from parental C312 are labeled to their right, whereas  
15 data point from the three transgenic lines are left unlabeled.) Note, however, that C312 does not shown any linear relationship because seed weight per seed shows little variability in the parental line. Therefore, the overall linear relationship among all the data points derives from the transgenic plants. The transgenic plants have more  
20 variability in and higher levels of delinted seed weight per seed and fiber weight per seed than parental C312 plants.

Figure 9 is a histogram of fuzz fiber weight per seed. It shows elevation in two of three transgenic lines, and a decrease in one transgenic line.

Figure 10 is a histogram of micronaire, which shows elevation in all three transgenic lines.

Figure 11 is a scatter plot of micronaire vs fiber weight per seed showing that  
25 these two parameters are interdependent at the 60% level. This is sensible since fiber weight per seed depends on 3 factors: number of fibers, length of fibers, and fiber wall thickness. Of these 3 factors, micronaire would depend only on fiber wall thickness. Note that this linear relationship also holds for C312, but the transgenics have higher  
30 values for fiber weight per seed and micronaire.

Figure 12 is a histogram of grams of force to break a single fiber (Tb; g). It shows elevation in all transgenic lines.

Figure 13 is a histogram of elongation to break a single fiber (% of original fiber length). It shows elevation in all transgenic lines. However, note that Elongation is highest in transgenic line 13-3a, which, among the transgenics, had the lowest increase in grams to break. This suggests that these two factors are primarily determined by different fiber properties, as would be predicted in theory and is confirmed by the scatter plots below.

Figure 14 is a histogram of work to break a single fiber ( $\mu\text{J}$ ). Work, which is a composite factor calculated from grams to break and elongation, is elevated in all transgenic lines.

Figure 15 is a scatter plot of grams of force to break a single fiber vs. micronaire. The graph shows an interdependency for these parameters over all data points of 68%. Both of these parameters would be expected to increase with a thicker fiber wall.

Figure 16 is a scatter plot of grams of force to break a single fiber vs. fiber weight per seed. These parameters are interdependent at a level of 61%, which is similar to the dependence on micronaire (See Figure 15). This supports the hypothesis that increased fiber weight per seed is due in large part to increased fiber wall thickness, since the two other parameters that can increase fiber weight per seed (increased fiber number and increased fiber length) would not be expected to increase grams to break.

Figure 17 is a scatter plot of work to break a single fiber vs. micronaire. These parameters are interdependent at a level of 48%. The intermediary level of dependency compared to grams to break and elongation alone (See Figure 19) is reasonable for this composite factor.

Figure 18 is a scatter plot of work to break a single fiber vs. fiber weight per seed. These parameters are interdependent at a level of 39%, which is similar to the dependence on micronaire (See Figure 17). As just described for Figure 16, this supports the hypothesis that increased fiber weight per seed is due in large part to increased fiber wall thickness.

Figure 19 is a scatter plot of elongation to break vs. micronaire. The graph shows that these parameters are not interdependent. Therefore, over-expression of SPS is predicted to enhance elongation by a mechanism independent of fiber wall thickness, which is consistent with theory.

Figure 20 is four overlaid scatter plots of photosynthetic rate vs. internal  $\text{CO}_2$  concentration for parental C312 growing in the Phytotron. Empty symbols are for two

plants growing at 30/15°C and filled symbols are for two plants growing at 30/28°C. All plants were assayed at 30°C. The graphs show that for parental C312, a previous cool night suppresses photosynthetic rate during the warm day.

Figure 21 is four overlaid scatter plots of photosynthetic rate vs. internal CO<sub>2</sub> concentration for the transgenic line 13-3a-1 growing in the Phytotron. Empty symbols are for two plants growing at 30/15°C and filled symbols are for two plants growing at 30/28°C. All plants were assayed at 30°C. The graphs show that for this transgenic line, a previous cool has no effect on the rate of photosynthesis during the next warm day.

Figure 22 is four overlaid scatter plots of photosynthetic rate vs. internal CO<sub>2</sub> concentration for the transgenic line 225-17a growing in the Phytotron. Empty symbols are for two plants growing at 30/15°C and filled symbols are for two plants growing at 30/28°C. All plants were assayed at 30°C. The graphs show that for this transgenic line, a previous cool has no effect on the rate of photosynthesis during the next warm day.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of controlling the cellulose synthesis in plants to optimize the level of production and quality of the products, in particular fiber, derived from the plants.

The word "fiber" is often used to unify a diverse group of plant cell types that share in common the features of having an elongated shape and abundant cellulose in thick cell walls, usually, but not always, described as secondary walls. Such walls may or may not be lignified, and the protoplast of such cells may or may not remain alive at maturity. Such fibers have many industrial uses, for example in lumber and manufactured wood products, paper, textiles, sacking and boxing material, cordage, brushes and brooms, filling and stuffing, caulking, reinforcement of other materials, and manufacture of cellulose derivatives. In some industries, the term "fiber" is usually inclusive of thick-walled conducting cells such as vessels and tracheids and to fibrillar aggregates of many individual fiber cells. Here the term "fiber" is used in its most inclusive sense, for example including: (a) thick-walled conducting and non-conducting cells of the xylem; (b) fibers of extraxylary origin, including those from phloem, bark, ground tissue, and epidermis; and (c) fibers from stems, leaves, roots, seeds, and flowers or inflorescences (such as those of *Sorghum vulgare* used in the manufacture of brushes



and brooms). In addition to wood from trees, cotton, and forage crops, the invention is applicable to all fibers, including, but not exclusively, those in agricultural residues such as corn, sugar cane, and rice stems that can be used in pulping, flax, hemp, ramie, jute, kenaf, kapok, coir, bamboo, spanish moss, abaca, and *Agave* spp. (e.g. sisal).

5 In a preferred embodiment, the invention provides a transgenic cotton plant wherein the transgenic cotton plant has an increased level of sucrose phosphate synthetase relative to a non-transgenic cotton plant. Table 1 shows the level of SPS activity from untransformed C312 plants and four transformed plant lines. All transformed plant lines show significant increases in SPS activity in both leaves and fiber.

10 Sucrose phosphate synthase plays a key role in the metabolic flux of carbon within plant cells. Genes encoding sucrose phosphate synthase have been isolated and sequenced from a number of plant species. [*Spinacia oleracea*: Salvucci et al., Plant Physiol., 102:529-536 (1993); Sonnewald et al., Planta, 189(2):174-181 (1993); *Oryza sativa*: Valdez-Alarcon et al., Gene, 170(2):217-222 (1996); *Craterostigma*  
15 *plantaquineum*: Ingram et al., Plant Physiol., 115(1):113-121 (1997); *Vicia faba*: Heim et al., Gene, 178(1-2):201-203 (1996); *Solanum tuberosum*: EMBL Accession No. X73477; *Citrus unshiu*: Akira et al., Mol. Gen. Genet., 252:346-351 (1996); *Saccharum officinarum*: Sugiharto et al., Plant Cell Physiol. 38:961-965 (1997); *Beta vulgaris*: Hesse et al., Mol. Gen. Genet., 247(4):515-520 (1995); *Zea mays*: Worrell et al., Plant  
20 Cell, 3:1121-1130 (1991); *Arabidopsis thaliana*, Bevan et al., NCBI Accession No. AL049487; *Synechocystis* sp.: Kaneko et al., DNA Res., 2(4):153-166 (1995); Kaneko et al., DNA Res., 3(3):109-136 (1996); and unknown organism: Van Assche et al., U.S. Patent No. 5,665,892-A, which are hereby incorporated by reference.] A comparison of several of the available SPS gene sequences from higher plants is provided  
25 in Figure 3. A comparison of a *Synechocystis* SPS (Kaneko et al., DNA Res., 2(4):153-166 (1995), which is hereby incorporated by reference) with the spinach SPS is provided in Figure 4; this protein from a cyanobacterium has as strong a homology with spinach SPS as all the higher plant proteins have among themselves. Preferred sucrose phosphate synthase genes include the genes isolated from spinach, *Arabidopsis*, beet, bean, citrus,  
30 maize, moss, potato, rice, sugar cane, and *Synechocystis*. The most preferred sucrose phosphate synthetase is spinach sucrose phosphate synthetase.

In addition to the known sequences of sucrose phosphate synthase, modifications of the known sequences are also within the scope of the invention. Variations in the

sequence including substitutions, insertions and deletions may be made to the known sequences of sucrose phosphate synthase. Comparisons of all the available sequences indicate which amino acids are highly conserved and those that are variable. Using that information, it is possible to choose variations that should still produce functional

5 proteins.

The maximum activity of sucrose phosphate-synthase may be determined colorimetrically according to the formation of sucrose-6-P (+ sucrose) from fructose-6-P and UDP-glucose by the method as described in (Copeland, "Enzymes of Sucrose Metabolism," Methods in Plant Biochemistry, 3:73-83 (1990), which is hereby

10 incorporated by reference). Frozen leaf or fiber tissue was pulverized under liquid nitrogen, then ground in 50 mM HEPES (pH 7.4), 10 mM MgCl<sub>2</sub>, 1 mM EDTA, 1 mM EGTA, 10% glycerol, and 0.1% Triton-X-100. A 28 µl aliquot of each supernatant was used in each SPS assay, and each extract was tested in triplicate. A 70 µl assay mixture contained 50 mM HEPES (pH 7.4), 10 mM UDPG, 6 mM fructose-6-P, 20 mM glucose-

15 6-P (an SPS activator), 10 mM MgCl<sub>2</sub>, 1 mM EDTA, 0.40 mM EGTA, 4.0% glycerol, and 0.04% Triton-X-100. The assay was conducted for 10 min at 32 – 34°C (on the plateau of maximal activity) then terminated by addition of 70 µl of 1N NaOH. Unreacted hexoses or hexose phosphates were destroyed by immersion of tubes in a boiling water bath for 10 min. After cooling to room temperature, 250µl of 0.1%

20 resorcinol in ethanol and 750 µl of concentrated HCl were added, followed by incubation for 8 min at 80°C. The tubes were quickly cooled to room temperature, A<sub>520 nm</sub> was measured in a spectrophotometer, and sucrose levels in plant extracts were determined in reference to a sucrose standard curve. Triplicate controls were made for each extract to normalize for possible different endogenous levels of sucrose in each extract. For

25 controls, NaOH was added to the assay tube before the plant extract was added; then these tubes were processed in parallel as above except for the step of assay termination by NaOH that was already done. Plant extracts were also analyzed for protein content by Bradford protein assay and leaf extracts were analyzed for chlorophyll content by its absorbance to allow comparison of SPS activities between different samples.

30 Alternatively, the activity of sucrose phosphate-synthase may be determined spectrophotometrically according to liberation of uridine-5'-diphosphate detected by a pyruvate-kinase coupling enzyme reaction as also described in (Copeland, "Enzymes of

Sucrose Metabolism," Methods in Plant Biochemistry, 3:73-83 (1990), which is hereby incorporated by reference).

In order to express the sucrose phosphate synthase in plants, transgenic plants carrying the gene encoding a sucrose phosphate synthase are produced by transforming a  
5 plant with a chimeric DNA construct that expresses sucrose phosphate synthase.

In order to express the sucrose phosphate synthase gene from the chimeric DNA, the construct should include a plant specific promoter. The promoter should ensure that the foreign gene is expressed in the plant. The promoter can be chosen so that the expression occurs only in specified tissues, at a determined time point in the plant's  
10 development or at a time point determined by outside influences. The promoter can be homologous or heterologous to the plant. Suitable promoters include e.g. the RUBISCO small subunit promoter, fiber-specific promoters, the promoter of the 35S RNA of the cauliflower mosaic virus described in U.S. Patent No. 5,034,322 (which is hereby incorporated by reference), the enhanced 35S promoter described in U.S. Patent  
15 No. 5,106,739 (which is hereby incorporated by reference), the dual S35 promoter, the FMV promoter from figwort mosaic virus that is described in U.S. Patent No. 5,378,619 (which is hereby incorporated by reference), the RI T-DNA promoter described in U.S. Patent No. 5,466,792 (which is hereby incorporated by reference), the octopine T-DNA promoter described in U.S. Patent No. 5,428,147 (which is hereby incorporated by  
20 reference), the alcohol dehydrogenase 1 promoter (Callis et al., Genes Dev., 1(10):1183-1200 (1987), which is hereby incorporated by reference), the patatin promoter B33 (Rocha-Sosa et al., EMBO J., 8:23-29 (1989), which is hereby incorporated by reference), the E8 promoter (Deikman et al., EMBO J., 7(11):3315-3320 (1988), which is hereby incorporated by reference), the beta-conglycin promoter (Tierney et al., Planta, 172:356-  
25 363 (1987), which is hereby incorporated by reference), the acid chitinase promoter (Samac et al., Plant Physiol., 93:907-914 (1990), which is hereby incorporated by reference), the *Arabidopsis* histone H4 promoter described in U.S. Patent No. 5,491,288 (which is hereby incorporated by reference), or the recombinant promoter for expression of genes in monocots described in U.S. Patent No. 5,290,924 (which is hereby  
30 incorporated by reference).

Preferred promoters include the RUBISCO small subunit promoter, the 35S promoters, fiber enhanced promoters, vascular cell enhanced promoters, stem cell enhanced promoters, or seed enhanced promoters. Such promoters may ensure

expression in a tissue specific or tissue-enhanced manner, but may allow expression in other cell types. For example it may ensure enhanced expression in photosynthetically active tissues (RUBISCO (Worrell et al., The Plant Cell, 3:1121-1130 (1991), which is hereby incorporated by reference)) or other mesophyll-cell-specific promoter (Datta et al., 5 Theor. Appl. Genet., 97:20-30 (1998), which is hereby incorporated by reference) or fibers (cotton-fiber-, xylem fiber-, or extra-xylary-fiber-specific or enhanced promoters). Other promoters can be used that ensure expression only in specified organs, such as the leaf, root, tuber, seed, stem, flower or specified cell types such as parenchyma, epidermal, or vascular cells. One example of a tissue specific promoter is the RB7 promoter that is 10 root specific (U.S. Patent No. 5,459,252, which is hereby incorporated by reference). Such promoters may be used either alone or in combination to optimize over-expression in the most desirable set of tissues or organs.

Preferred cotton fiber-enhanced promoters include those of the cotton fiber-expressed genes E6 (John et al., Plant Mol. Biol., 30:297-306 (1996) and John et al., Proc. Natl. Acad. Sci., 93:12768-12773 (1996), which are hereby incorporated by reference), 15 H6 (John et al., Plant Physiol., 108:669-676, (1995), which is hereby incorporated by reference), FbL2A (Rinehart et al., Plant Physiol., 112:1331-1341 (1996) and John et al., Proc. Natl. Acad. Sci. USA, 93:12768-12773 (1996), which are hereby incorporated by reference), rac (Delmer et al., Mol. Gen. Genet., 248:43-51 (1995), which is hereby incorporated by reference); CeaA (Pear et al., Proc. Natl. Acad. Sci. USA, 93:12637-12642 20 (1996), which is hereby incorporated by reference); CAP (Kawai et al., Plant Cell Physiol. 39:1380-1383 (1998)); ACP (Song et al., Biochim. Biophys. Acta 1351:305-312 (1997); and LTP (Ma et al., Biochim. Biophys. Acta 1344:111-114 (1997)).

Preferred promoters enhancing expression in vascular tissue include the CAD 2 promoter (Samaj et al., Planta, 204:437-443 (1998), which is hereby incorporated by 25 reference), the Pt4Cl1 promoter (Hu et al., Proc. Natl. Acad. Sci. USA, 95:5407-5412 (1998), which is hereby incorporated by reference), the C4H promoter (Meyer et al., Proc. Natl. Acad. Sci. USA, 95:6619-6623 (1998), which is hereby incorporated by reference), the PtX3H6 and PtX14A9 promoters (Loopstra et al., Plant Mol. Biol., 27:277-291 30 (1995), which is hereby incorporated by reference), the RolC promoter (Graham, Plant Mol. Biol., 33:729-735 (1997), which is hereby incorporated by reference), the Hvhsp17 promoter (Raho et al., J. Expt. Bot., 47:1587-1594 (1996), which is hereby incorporated

by reference), and the COMT promoter (Capellades et al., Plant Mol. Biol., 31:307-322 (1996), which is hereby incorporated by reference).

Preferred promoters enhancing expression in stem tissue include pith promoters (Datta, Theor. Appl. Genet., 97:20-30 (1998) and Ohta et al., Mol. Gen. Genet., 225:369-378 (1991), which are hereby incorporated by reference), and the anionic peroxidase promoter (Klotz et al., Plant Mol. Biol., 36:509-520 (1998), which is hereby incorporated by reference). Preferred promoters enhancing expression in phloem, cortex and cork, but not xylem or pith, include the Psam-1 promoter (Mijnsbrugge et al., Plant and Cell Physiol., 37:1108-1115 (1996), which is hereby incorporated by reference).

Preferred promoters enhancing expression in seeds include the phas promoter (Geest et al., Plant Mol. Biol. 32:579-588 (1996)); the GluB-1 promoter (Takaiwa et al., Plant Mol. Biol. 30:1207-1221 (1996)); the gamma-zein promoter (Torrent et al. Plant Mol. Biol. 34:139-149 (1997)), and the oleosin promoter (Sarmiento et al., The Plant Journal 11:783-796 (1997)).

Truncated or synthetic promoters including specific nucleotide regions conferring tissue-enhanced expression may also be used, as exemplified by identification of regulatory elements within larger promoters conferring xylem-enhanced expression (Seguin et al., Plant Mol. Biol., 35:281-291 (1997); Torres-Schumann et al., The Plant Journal, 9:283-296 (1996); and Leyva et al., The Plant Cell, 4:263-271 (1992), which are hereby incorporated by reference).

In one embodiment of the invention the chimeric DNA construct is stably integrated into the genome of the cotton plant. When a plant is transformed by *Agrobacterium* mediated transformation, a portion of the Ti plasmid integrates into the plant genome and is stably passed on to future generations of plant cells.

Numerous methods exist for transforming plant cells. The preferred methods include electroporation, *Agrobacterium* mediated transformation, biolistic gene transformation, chemically mediated transformation, or microinjection.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA (Crossway, Mol. Gen. Genetics, 202:179-185 (1985), which is hereby incorporated by reference). The genetic material may also be transferred into the plant cell using polyethylene glycol (Krens et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference).

Another approach to transforming plant cells with a gene that increases fiber and seed yield and fiber quality is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies (Fraley et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference).

The DNA molecule may also be introduced into the plant cells by electroporation (Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the

bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome (Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference).

After transformation, whole transformed plants can be recovered. If transformed seeds were produced directly, these can be selected by germination on selection medium and grown into plants (Glough et al. The Plant Journal 16:735-743 (1998), which is hereby incorporated by reference). If transformed pollen was produced directly, this can be used for *in vivo* pollination followed by selection of transformed seeds (Touraev et al., The Plant Journal 12:949-956 (1997), which is hereby incorporated by reference). If meristems were transformed, these can be grown into plants in culture then transferred to soil (Gould, J. et al., Plant Cell Rep. 10:12-16 (1991), which is hereby incorporated by reference).

If protoplasts or explants were transformed, plants can be regenerated. Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1, New York, New York:MacMillan Publishing Co., (1983); and Vasil, ed., Cell Culture and Somatic Cell Genetics of Plants, Orlando:Acad. Press, Vol. I (1984), and Vol. III (1986), which are hereby incorporated by reference. Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, species of sugarcane, sugar beets, cotton, forest trees, forage crops, and fiber producing plants. Regeneration is also possible in seed-producing plants including, but not limited to, maize, rice, wheat, soybean, rape, sunflower, and  
5 peanut.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be  
10 cultivated in accordance with conventional procedure with the presence of the gene encoding the sucrose phosphate synthase resulting in enhanced seed yield and/or enhanced fiber yield and/or enhanced fiber quality. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants.

15 The present invention also provides seeds produced from the transgenic plant having increased synthesis of sucrose phosphate synthase.

In another embodiment, the invention provides a method of increasing the yield of cotton plant by introducing into a cotton plant a chimeric DNA construct that alters sucrose phosphate synthase activity in an amount sufficient to increase the yield of the  
20 cotton plant. A chimeric gene may be introduced into plant cells or tissue. Transformed cells are selected, usually by the use of a selectable marker. The transformed cells are then used to generate a transformed plant (Fraley et al., Proc. Natl. Acad. Sci. USA, 79:1859-1863 (1982), which is hereby incorporated by reference).

Preferred plants are cotton plants. The transformed plants may have an increase in  
25 the yield of cotton seeds or cotton fiber.

The present invention also provides a method of increasing the quality of cotton fiber produced from a cotton plant by introducing into a cotton plant a chimeric DNA construct that alters the sucrose phosphate synthase activity in an amount sufficient to increase the quality of the cotton fiber produced by the cotton plant.

30 The level of sucrose phosphate synthase may be increased by expressing factors that increase the level of expression of the gene. Such factors may act on regulatory sites controlling expression that are normally located near the sucrose phosphate synthase gene or heterologous regulatory sites located near the gene in a chimeric construct.



Alternatively, the level of sucrose phosphate synthase may be increased by introducing a chimeric DNA construct that directly expresses a sucrose phosphate synthase.

Generally, the present invention can be used to change the ratio of cellulose to the dry weight of the whole plant or to the dry weight of plant components by introducing  
5 into a plant a chimeric DNA construct capable of altering sucrose phosphate synthase activity in an amount sufficient to change the ratio of cellulose to the dry weight of the whole plant or plant components. The change in cellulose can be observed in relation to total weight of the plant or fractionated parts of plants including, but not exclusively, starch, total cell walls, cell wall of fibers, particular organs such as stems, or cell wall  
10 components such as pectins, hemicelluloses, proteins, extractives, and lignin. The change in the ratio of cellulose to the fractionated parts of plants can be observed when the fractionated parts are considered alone or in any additive combination.

Changes in qualities as claimed in this invention refer to changes of at least 10% compared to a plant lacking the transgene. For example, the ratio of cellulose in cell  
15 walls may be changed from 20% to 18% or lower or 22% or higher. Such change compared to parental level could apply to all cell walls or any cell wall fraction of a plant.

In a preferred embodiment, the dry weight of cellulose may be increased so that its ratio to other dry weight components exceeds 40%. Such increase to exceed 40% could apply to wood, fibers, and other cellulose-rich cell walls such as collenchyma and  
20 thickened xylem parenchyma.

To accomplish certain changes, the level of sucrose phosphate synthase may be decreased by expressing factors that decrease the level of expression of the gene. Such factors may act on regulatory sites controlling expression that are normally located near the sucrose phosphate synthase gene or heterologous regulatory sites located near the  
25 gene in a chimeric construct. Alternatively, in anti-sense technology, the level of sucrose phosphate synthase may be decreased by introducing a chimeric DNA construct that contains the complementary cDNA of a sucrose phosphate synthase (Arndt et al., Genome, 40:785-797 (1997), which is hereby incorporated by reference). Alternatively, decreased SPS activity might be induced by homology dependent gene silencing  
30 (Wassenegger et al. Plant Mol. Biol. 37:349-362 (1998), which is hereby incorporated by reference), virus-induced gene silencing (Baulcombe, Curr. Op. Plant Biol. 2:109-113 (1999), which is hereby incorporated by reference), chimeric RNA/DNA oligonucleotides (Zhu et al., Proc. Natl. Acad. Sci. USA 15:8768-8773 (1999), which is hereby

incorporated by reference), or homologous recombination (Shalev et al. Proc. Natl. Acad. Sci. USA 96:7398-7402 (1999), which is hereby incorporated by reference).

In yet another embodiment, the invention provides a method of increasing tolerance of photosynthetic efficiency to cool night temperatures by introducing into a  
5 plant a chimeric DNA construct capable of altering sucrose phosphate synthase activity in an amount sufficient to increase tolerance of photosynthetic efficiency to cool night temperatures.

The present invention can be used to regulate the thickness of cell walls in a plant by introducing into the plant a chimeric DNA construct that will change the sucrose  
10 phosphate synthase activity. In particular, the method can be used to increase the yield of harvestable fiber from any fiber producing plant.

In a preferred embodiment, the plant is a fiber producing plant. More preferred fiber producing plants are sugarcane, sugar beets, forest trees, forage crops, fiber producing plants, and seed producing plants.

15 In yet another embodiment, the present invention can be used to increase the harvestable yield of fiber from a plant. The invention may also be used to alter the quality of fiber isolated from the plant... Changes in sucrose phosphate synthase can change fiber strength, fiber length, or weight per unit length. Changes may either increase or decrease the strength, length or weight per unit length.

20 The present invention can be used to increase the yield of seed harvested from a seed producing plant by introducing into the plant a chimeric DNA construct that will increase the sucrose phosphate synthase activity.

The methods of the invention are broadly applicable and can be used in a wide variety of plants including cotton, forest trees, forage crops, beets, flax, hemp, jute, and  
25 other fiber-producing plants. They can also be used in seed producing plants including cotton, flax, wheat, rice, corn, soybean, Brassica sp. (e.g. rape), sunflower, safflower, peanut, palm, and other seed producing plants.

The methods of the invention are further described in the examples that follow.

## EXAMPLES

### Example 1 – Materials and Methods

5           Most plants described were grown in one chamber at the Duke University  
Phytotron: 360 ppm (normal) CO<sub>2</sub>; 30°/15-19°C day/night cycle; 14h day/10h night; 1200  
μmol m<sup>-2</sup>s<sup>-1</sup> (metal halide) illumination; irrigation 2x daily with 1/2 strength Hoagland's  
solution; potted in a mixture of gravel and sand in 4 gallon pots. A change to 30/19°C  
10   the maturation of first bolls in C312 and all transgenic lines. This temperature condition  
is subsequently referred to as 30/15°C for simplicity. This chamber is emphasized  
because its temperature and CO<sub>2</sub> conditions represent those likely to be encountered by  
cotton crops in the field, for example but not exclusively on the Texas Southern High  
Plains.

15           Other plants were grown in the Duke University Phytotron in 3 other chambers as  
described except with the following changes: (a) 360 ppm CO<sub>2</sub>, 30°/28°C day/night  
cycle; (b) 700 ppm (elevated) CO<sub>2</sub>, 30°/15-19°C day/night cycle; and (c) 700 ppm CO<sub>2</sub>,  
30°/28°C day/night cycle.

          Other plants were grown in the Texas Tech University greenhouse: natural CO<sub>2</sub>  
20   and illumination; approximately 32/22°C day/night cycle; 2 gallon pots; irrigation 2-3x  
daily; slow-release fertilizer in the soil and soluble fertilizer applied 1x weekly.

          All open bolls were harvested from each plant from which seed and fiber  
parameters were evaluated. Lint fiber was removed from the seeds by hand-stripping.  
Cotton seeds are covered with lint fiber (the long fiber used for textiles) and fuzz fiber  
25   (short fibers used in various industrial applications). (Lint) fiber weight and fuzzy seed  
weight from each plant was determined by weighing. Hereafter, 'fiber' refers to lint  
fiber, with fuzz fiber specified when necessary. Seed number per plant was determined  
by counting. (Seeds and fiber of underdeveloped "motes" were not included.) Fiber was  
sent to Cotton Incorporated, Raleigh, NC for HVI, AFIS, and Mantis fiber quality  
30   analysis. Seeds from the 30/15°C chamber were subsequently acid-delinted, air-dried,  
and weighed. From this chamber, fuzz fiber weight per seed was determined by  
subtraction of the weights of fuzzy and delinted seeds.

For plants for which stem weight was determined, any unopened bolls and leaves and petioles were removed. Above-ground stems were oven-dried and weighed.

The plant line used is a Coker 312 wild-type (untransformed parent) and four transgenic lines. Transgenic plant lines, each known to represent separate transformation events, are designated 13-3a, 225-17a, 40-4b, and 40-6a. T0, T1, or T2 represent primary transformants and the first and second filial generations, respectively. All transgenic plants tested were Kanamycin resistant as determined from formation of lateral roots of germinating seedlings within agar containing Kanamycin. The segregation ratio of seeds germinated on kanamycin is expressed as resistant/sensitive ratio (Table 1). Ratios were assessed after 7 - 14 days to include most slow-germinating seeds.

The number of individual plants grown in the Phytotron to yield average data for each parameter (except for 40-6a-4) is indicated as Phytotron Plants (n) (Table 2). Line 40-6a-4, although it generally performed consistently with the other lines, was omitted from fiber quality averages because it was represented by only one plant in the 30/15°C, 360 ppm CO<sub>2</sub> chamber. Values from two T2 lineages of line 40-4b were averaged together because T1#1 and T1#4 are similar siblings (except for segregation ratio) that generated similar T2 progeny.

Leaf and fiber RNA levels were determined by Northern analysis of the mRNA for foreign SPS in the leaf, scored as positive or negative (Table 1). Extractable SPS activity (production of sucrose) is standardized as  $\mu\text{mol sucrose/mg chlorophyll/hour}$  for leaf activity or as  $\mu\text{mol sucrose/mg protein/hour}$  for fiber activity (Table 1).

The Boll # per Plant is the number of non-aborted bolls on each plant.

The Delinted Seed Weight per Seed (g) and (Lint) Fiber Weight per Seed (g) (Table 2) are data derived from all open bolls of each plant at the time the experiment was terminated. Under 30/28°C, all bolls had opened, but under 30/15°C, some unopened bolls were left on each plant at termination. Each data point represented 192 - 487 seeds yielding 24.5 - 48.5 g lint fiber.

Bulk (or bundle) fiber properties as determined by automated HVI and AFIS testing are summarized in Tables 3 and 4. The fiber micronaire (by HVI) is a unitless measurement that depends both on fiber maturity (or wall thickness determined by secondary wall cellulose content) and fiber diameter.

Fiber bundle strength (by HVI) is expressed in units of (cN/tex). It is the specific strength of the fiber bundle is which the individual fiber fineness (tex) is calculated from the Micronaire value.

Fiber fineness (by AFIS) is expressed as (mTex). It represents the weight, in  
5 milligrams, of one kilometer of the fiber. One thousand meters of fibers with a mass of 1 milligram equals 1 millitex.

The fiber maturity ratio (by AFIS) is an expression of the degree of cell wall thickening (depending on secondary cell wall cellulose deposition). It is the ratio of fibers with a 0.5 (or more) circularity ratio divided by the amount of fibers with 0.25 (or  
10 less) circularity. (Fibers with thicker walls are less prone to collapse and remain more circular upon drying.) The higher the maturity ratio, the more mature the fibers are and the better the fibers are for dyeing.

The immature fiber content ("IFC%", by AFIS) is the percentage of fibers with less than 0.25 maturity. The lower the IFC%, the more suitable the fiber is for dyeing.

Several different units are used as indicators of fiber length. Table 3 shows values  
15 for three of these as now described. Upper half mean ("UHM", by HVI) is the mean length of the longest one half of the fibers (weight biased). The fiber Uniformity Index ("UI", by HVI) expresses the ratio of the mean value (Mean Length) to the Upper Half Mean Length. It is a measure of the fiber length scatter within the population; if all fibers  
20 were the same length UI would equal 100%. Short Fiber Content ("SFC %", by HVI) is the percentage of fibers less than 1/2" long on a weight basis. HVI is thought to measure Short Fiber Content as determined by genetics only since the measurement does not impose additional potential fiber breaking stress.

Other fiber length indicators discussed in the text are as follows. The weight basis  
25 length ("L(w)" [in], by AFIS) is the average length of fibers calculated on a weight basis. The number basis length ("L(n)" [in], by AFIS) is the mean length of fibers calculated by number. The length "L5% (n)" [in] (by AFIS) is the 5% span length, or the length spanned by 5% of the fibers when they are parallel and randomly distributed. The length  
"L2.5% (n)" [in] (by AFIS) is the 2.5% span length, or the length spanned by 2.5% of the  
30 fibers when they are parallel and randomly distributed. The "UQL (w)" [in] (by AFIS) is the upper quartile length of fibers by weight, or the length exceeded by 25% of the fibers by weight. Finally, the "SFC (n)" [in] and "SFC (w)" [in] (by AFIS) are the percentage of fibers less than 0.50 inches long on a number and weight basis, respectively. In

contrast to HVI, AFIS beats the fibers before taking these measurements, which has potential to cause fiber breakage. Therefore, AFIS SFC values are a good indication of the characteristics of the fiber after normal processing.

Single fiber strength and elongation parameters derived from Mantis testing are summarized in Table 5. "Tb" [g] is grams of force to break a single fiber. "Elongation" [%] is single fiber elongation before break as % of original length. "Work" [ $\mu$ J] is a composite of Tb and Elongation, representing the work expended to break a single fiber.

Detailed methods for particular experiments are included under the Examples.

10 **Example 2 - Summary of Results Demonstrating Increased Fiber and Seed Yield in Transgenic Plants with Increased SPS Activity**

Transgenic cotton plants with spinach SPS under the control of a constitutive promoter showed foreign gene expression in the leaf and fiber as demonstrated by Northern analysis. At the T1/T2 generation, they showed average increased SPS enzyme activity of 3.3 times and 2.3 times in the leaf and fiber, respectively, compared to parental C312 (Table 1). In this and all following tables, values indicating superior features of transgenic plants compared to parental C312 are shown in bold.

**Table 1**  
**Characterization of Spinach SPS gene expression and**  
**Total SPS Activity in Transgenic Plants**

Plant Line	Segregation Ratio	Leaf RNA	Fiber RNA	Leaf SPS Activity (chlorophyll)	Normalized Leaf SPS Activity	Fiber SPS Activity (protein)	Normalized Fiber SPS Activity
C312-wt	na	-	-	23.53 <sup>a</sup>	1.0	39.91	1.0
				31.30 <sup>b</sup>	1.0		
13-3a							
T0		+		119.2	5.1		
T1	22:6						
T1#1@T2	66:0		+	127.2	4.0	103.39	2.6
225-17a							
T0		+		118.5	5.0		
T1	25:12		+	121.8	3.9	93.71	2.4
40-4b							
T0		+		107.3	4.6		
T1	11:4						
T1#1@T2	51:16			60.3	1.9	91.67	2.3
T1#4@T2	10:0		+	66.4	2.1	76.00	1.9
40-6a							
T0		+		89.3	3.8		
T1	6:5						
T1#4@T2	9:2			57.6	1.8	74.12	1.9
Transgenic Average at T1/T2 <sup>c</sup>				103.9	3.3	85.4	2.3

<sup>a</sup> Value measured and used for T0 comparisons.

<sup>b</sup> Value measured and used for T1 and T2 comparisons.

<sup>c</sup> Excludes values for line 40-6a and uses a composite average value for line 40-4b to parallel the procedures used in analysis of fiber quality data.

Over the first 9 weeks of growth in the 30/15°C, 360 ppm CO<sub>2</sub> Phytotron chamber during which plant height and leaf number were measured, the transgenic lines grew similarly to parental C312. The average height of the transgenic plants was 0.90 x the value for parental C312. The average leaf number of the transgenic plants was 1.02 x parental C312.

In the 30/15°C, 360 ppm CO<sub>2</sub> Phytotron chamber, up-regulated SPS gene expression caused increases in yield components of the fiber and seed crop (Table 2).

Table 2

**Yield Components of SPS Transgenic Plants Compared to Parental C312 (at 30/15°C and 360 ppm CO<sub>2</sub>)**

5

Plant Line	Phyto-tron Plants (n)	Boll # per Plant	Normal-ized Boll #	Delinted Seed Weight per Seed (g)	Normal-ized Seed Weight per Seed	Fiber Weight per Seed (g)	Normal-ized Fiber Weight per Seed
<b>C312-wt</b>	4	22.8	1.0	0.090	1.0	0.047	1.0
<b>13-3a</b>							
T1#1@T2	4	26.5	1.16	0.107	1.19	0.058	1.23
<b>225-17a</b>							
T1	4	26.0	1.14	0.110	1.22	0.063	1.34
<b>40-4b</b>							
T1#1&#4@T2	5	28.2	1.24	0.100	1.11	0.057	1.21
<b>40-6a</b>							
T1#4@T2	1	28.0	1.23	0.105	1.17	0.054	1.15
<b>Transgenic Average at T1/T2<sup>a</sup></b>		26.9	1.18	0.106	1.18	0.059	1.25

<sup>a</sup>Average omits line 40-6a because of few replications.

Both cotton fiber and cotton seeds are valuable crops, the lint fibers for use in textiles and other applications and the seeds as a source of oil and seed meal. In addition, short fuzz fibers (also called linters) are harvested as a source of chemical cellulose, among other uses. Increases were observed in number of bolls per plant, seed weight per seed, fiber weight per seed, and fuzz fiber weight per seed. Boll number per plant indicates overall capacity for production of seeds with attached fiber. Furthermore, increased weight of seed and fiber per seed generates increased yield. Transgenic plants over-expressing SPS achieve increased yield of two types of crops at the same time: seed yield based primarily on storage of protein and oil and fiber yield based on storage of cellulose. Therefore, plants that over-express SPS can be predicted to generate more income per acre for the cotton producer based on crop yield alone. Coker 312 plants over-expressing SPS can also be used for future transformations to help overcome any potential yield drag from use of this old cultivar in genetic engineering. Seed and fiber



yield can be maximized at the same time in other crop plants, and stiffer stems can be generated to resist lodging without sacrifice of seed yield.

**Increased Boll Number per Plant:**

5           Three transgenic lines tested in the 30/15°C, 360 ppm CO<sub>2</sub> chamber with good replication showed 14 - 24% increase in boll number per plant compared to parental C312, with an average increase of 18% (Table 2). Increased boll number of all transgenic lines was also observed in the 30/15°C, 700 ppm CO<sub>2</sub> and 30/28°C, 700 PPM CO<sub>2</sub>  
10   chambers.

**Increased Fiber Weight per Seed:**

          Three transgenic lines tested in the 30/15°C, 360 ppm CO<sub>2</sub> chamber showed 21 -  
15   34% increase in fiber weight per seed compared to parental C312, with an average increase of 25% (Table 2, Fig. 5). This effect was not consistently observed in other chambers. Fiber weight per seed is a composite of fiber number, fiber length, and fiber wall thickness. Since average fiber micronaire (indicating increased wall thickness) and other related factors do increase in all transgenic lines across all chambers (see below),  
20   one may infer that unmeasured factors such as changing fiber number might impact fiber weight per seed under nearly constant warm temperature or elevated CO<sub>2</sub>.

          A measurement sometimes taken in lab-based yield analysis is "lint %" = (lint fiber weight)/(total seed and lint fiber weight). This parameter increases 1.8 – 2.7% for three transgenic lines above the parental C312 value of 31.14% (average increase for  
25   transgenics of 2.1 %). This value under-estimates fiber yield improvement in transgenic lines because seed weight also increases (see below).

**Increased Seed Weight per Seed:**

30           Three transgenic lines tested in the 30/15°C, 360 ppm CO<sub>2</sub> chamber showed 11 - 22% increase in delinted seed weight per seed compared to parental C312, with an average increase of 18% (Table 2, Fig. 6). Only fuzzy seeds have been weighed from other chambers. However, comparing fuzzy and delinted values from the 30/15°C, 360 ppm CO<sub>2</sub> chamber indicates that fuzzy seed values are representative of the trends in seed  
35   yield. Fuzzy seeds showed increased seed weight per seed in the transgenic lines growing

in the other three chambers with only one exception (225-17a showed seed weight per seed equal to parental C312 in the 30/28°C, 700 ppm CO<sub>2</sub> chamber).

The ratio of Fiber Weight per Seed to Delinted Seed Weight per Seed in the 30/15°C, 360 ppm CO<sub>2</sub> chamber was increased by an average of 9.0% in three transgenic lines (Fig. 7). A scatter plot of fiber weight per seed vs. delinted seed weight per seed shows that transgenic plants separate from parental C312 through increases in both of these yield components together (Fig. 8). However, there is preferential enhancement of fiber weight compared to seed weight in SPS transgenic plants.

#### 10 **Increased Fuzz Fiber Weight per Seed:**

Fuzz fiber weight per seed was obtained by subtracting the unit seed weight of delinted seed from the unit seed weight of fuzzy seeds from the 30/15°C, 360 ppm CO<sub>2</sub> chamber (Fig. 9). Two transgenic lines (225-17a and 40-4b) showed increases (averaging 15 19% increase compared to parental C312) and one transgenic line (13-3a) showed a decrease (19% decrease compared to parental C312). Seeds of line 13-3a also looked blacker before delinting, suggesting initiation of fewer fuzz fibers than on seeds of either parental C312 or the other two transgenic lines. Therefore, transgenic lines show some variation in numbers of fuzz fibers initiated, but, once initiated, over-expressed SPS 20 enhances their yield similarly to lint fibers.

#### **Example 3 - Summary of Results Demonstrating Increased Fiber Quality as Analyzed by Automated HVI and AFIS on Bulk Samples**

25 Many spinning properties of cotton depend on its properties as a bulk sample. HVI and AFIS are automated systems that analyze these properties, yielding complementary information. These analyses show that the quality parameters of fiber produced by SPS transgenic plants are moving as a set into the premium quality range. Fiber from SPS transgenic plants is longer, stronger, and more mature—all these features 30 are currently valued by the cotton processing and textile industries to make high quality fabrics. Even under a stressful 30/15-19°C temperature cycle typical of the Texas Southern High Plains, the quality of fiber from SPS transgenic plants resembles that of premium cotton such as is traditionally grown in California. Therefore, cotton fiber from SPS transgenic plants can serve an expanded set of end-use markets and sell for a

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premium price. Producers growing SPS transgenic cotton should also be able to avoid price discounts for inferior quality such a low micronaire that can result from traditional cotton grown on the Texas Southern High Plains. Therefore, SPS transgenic cotton should stabilize or enhance income per acre for the cotton producer based on improved  
 5 fiber quality.

#### **Improvements Under 30/15°C, 360 ppm CO<sub>2</sub>:**

Key bulk fiber quality parameters from fiber grown in the 30/15°C, 360 ppm CO<sub>2</sub>  
 10 chamber and analyzed by HVI and AFIS are shown in Table 3. Factors of increase for transgenic lines over parental C312 are shown in Table 4.

**Table 3**

**Fiber Quality Parameters of SPS Transgenic Plants Compared to Parental C312  
 (at 30/15°C and 360 ppm CO<sub>2</sub>)**

Plant Line	Phyto-tron Plants (n)	Fiber Micro-naire	Fiber Bundle Strength (cN/tex)	Fiber Finess (mTex)	Fiber Matur-ity Ratio	Immature Fiber Content (%)	Fiber Length (UHM) (in)	Fiber Uniform-ity (UI, %)	Short Fiber Content (% by HVI)
C312-wt	4	3.68	27.1	167	0.89	7.45	1.04	83.1	7.5
13-3a									
T1#1@T2	4	4.55	28.8	170	0.92	6.85	1.15	88.9	5.9
225-17a									
T1	4	5.12	31.0	189	0.99	4.35	1.14	87.9	2.9
40-4b									
T1#1&#4@T2	5	4.50	31.1	180	0.95	5.64	1.12	84.8	5.9
40-6a									
T1#4@T2	1	5.30	29.6	177	0.96	5.20	1.08	86.1	11.3
Transgenic Average at T1/T2 <sup>a</sup>		4.72	30.3	180	0.95	5.61	1.14	87.2	4.9

<sup>a</sup> Average omits line 40-6a because of few replications.

Table 4

**Changes in Fiber Quality Parameters of SPS Transgenic Plants  
(at 30/15°C and 360 ppm CO<sub>2</sub>)**

- 5 (Values are shown normalized to C312-wt values set to 1.0 or as % changes from parental C312 values.)

Plant Line	Phyto-tron Plants (n)	Normal-ized Fiber Micro-naire	Normal-ized Fiber Bundle Strength (cN/tex)	Normal-ized Fiber Fine-ness (mTex)	Normal-ized Fiber Maturity Ratio	Change in Immature Fiber Content (%)	Normal-ized Fiber Length (UHM)	Change in Fiber Uniformity (UI, %)	Change in Short Fiber Content (% by HVI)
C312-wt	4	1.00	1.00	1.00	1.00	7.45%	1.00	83.1%	7.5%
13-3a									
T1#1@T2	4	1.23	1.06	1.02	1.03	-0.60%	1.11	+5.8%	-1.6%
225-17a									
T1	4	1.39	1.14	1.13	1.11	-3.10%	1.09	+4.8%	-4.6%
40-4b									
T1#1&#4@T2	5	1.22	1.15	1.08	1.07	-1.81%	1.07	+1.7%	-1.6%
40-6a									
T1#4@T2	1	1.44	1.09	1.08	1.08	-2.25%	1.04	+3.0%	+3.8%
Transgenic Average Changes at T1/T2 <sup>a</sup>		1.28	1.12	1.08	1.07	-1.84%	1.10	+4.1%	-2.6%

<sup>a</sup> Average omits 40-6a because of few replications.

10

Micronaire. Three transgenic lines showed an average increase of 28% to attain an average micronaire of 4.72 (Fig. 10). Micronaire depends on secondary wall thickness and fiber diameter. It is desirable that increases in micronaire occur because of increased secondary wall thickness, not because of increased fiber diameter. The fiber diameter is estimated from the standardized relationship between Fiber Fineness and Fiber Maturity Ratio (Table 3) and found to be little-changed in transgenic lines. Both parental C312 and the transgenic lines had estimated fiber diameter between 16.5 - 17.0  $\mu\text{m}$ . Furthermore, a plot of Micronaire vs. Fiber Weight per Seed shows an interdependence at the 59% level (Fig. 11), supporting the existence of thicker walls in fibers of SPS transgenic plants. Other data on fiber strength, maturity ratio, and immature fiber content (see below) also support an increase in wall thickness of fiber from SPS transgenic plants. Over 90% of the thickness of the cotton fiber wall is due to deposition of almost pure

20

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cellulose in the secondary cell wall. Therefore, over-expression of SPS has increased the cellulose content of cotton fibers.

Fiber Bundle Strength. Three transgenic lines showed an average increase of 12% to attain an average bundle strength of 30.3 cN/tex.

5 Fiber Fineness. Three transgenic lines showed an average increase of 8% to attain an average fineness of 180. Higher fiber fineness is traditionally undesirable because it is usually attributed to larger fiber diameter. However, since fiber of SPS transgenic plants has diameter approximately equal to parental C312 (see above), the increased fineness is likely attributable to increased fiber wall thickness yielding more weight per unit length.

10 Therefore, increased fineness of fiber from SPS transgenic plants is expected to be a neutral or positive fiber quality factor.

Fiber Maturity Ratio. Three transgenic lines showed an average increase of 7% to attain an average maturity ratio of 0.95, which falls in the "above average" range (0.95 - 1.00). This is superior to parental C312 with its average value of 0.89 in the "mature" range (0.85 - 0.95).

Immature Fiber Content. Three transgenic lines showed an average decrease of 1.84% to attain an average of 5.61% immature fibers. Transgenic fibers are superior to those of parental C312, which contain an average of 7.45% immature fibers.

Fiber length. Three transgenic lines showed an average increase in Upper Half  
20 Mean length of 10% to attain average UHM of 1.14 inches. The three lines also have more uniform fiber length, with average Uniformity Index increased 4.1% to attain average UI of 87.2%. The three lines also have fewer short fibers, with average Short Fiber Content by HVI decreasing 2.6% to attain average SFC% of 4.9 %. In addition to data summarized in Tables 3 and 4, other AFIS parameters support increased fiber length  
25 in fibers of SPS transgenic plants. For the average of three transgenic lines, L(w) increases 7% to 1.06 inches, L(n) increases 9% to 0.96 inches, UQL (w) increases 6% to 1.19 inches, L5% (n) [in] increases 6% to 1.34 inches, and L2.5% (n) increases 5% to 1.46 inches. Similarly, AFIS showed that on average three transgenic lines had decreased short fiber content with SFC% (w) decreasing 1.0% to 3.1% and SFC% (n) decreasing  
30 2.0% to 10.6%. (These AFIS SFC% averages omit the values from one plant of line 40-4b because they were extreme outliers that greatly skewed the averages away from the values for the other four plants in the line.) Since AFIS beats the fibers before taking the

measurement, these reduced SFC% values are good indications for improved utility of fibers from SPS transgenic plants in normal fiber processing.

**Improvements Under Diverse Environmental Conditions:**

5

Many fiber quality parameters were enhanced most for transgenic lines compared to parental C312 in the 30/15°C, 360 CO<sub>2</sub> ppm chamber, which was the only typical growing condition for cotton tested. However, fiber quality was also maintained or enhanced in transgenic plants growing in the other Phytotron chambers where

10 temperature was varied from 30/15°C to 30/28°C and/or CO<sub>2</sub> was varied from 360 ppm to 700 ppm. This is demonstrated by transgenic values and change from values for C312 of fiber quality data from the three transgenic lines growing in the other three chambers averaged together, excluding the 30/15°C, 360 ppm chamber that has been summarized independently. Over-expression of SPS maintains especially strong effects on Micronaire  
15 and average fiber length, L(n), with parallel consistent effects on UI and SFC.

Micronaire. 4.65; 1.13x compared to the C312 average value.

Fiber Bundle Strength. 30 cN/tex; 1.02x.

Fiber Maturity Ratio. 0.92, 1.03x.

Immature Fiber Content. 6.69%; decreased 1.1%.

20 Length (n). 0.95 inches; 1.08x.

Upper Quartile Length. 1.21 inches; 1.03x.

Fiber Uniformity Index. 87.7%; increased 1.3%.

Short Fiber Content (w) by HVI. 3.77%; decreased 1%.

Short Fiber Content (w) by AFIS. 3.95%; decreased 1.75%.

25

Changes within each plant line are compared in average values for the quality parameters of Micronaire, UHM, UI, bundle strength, SFC%, UQL, L(n), IFC%, and maturity ratio when 30/15°C changed to 30/28°C (at 360 ppm CO<sub>2</sub>) or 360 ppm CO<sub>2</sub> changed to 700 ppm CO<sub>2</sub> (at 30/15°C). These calculations show that over-expression of  
30 SPS in transgenic lines promotes nearly maximum increases in fiber quality even at the most limiting 30/15°C, 360 ppm CO<sub>2</sub> condition. In contrast, raising the minimum temperature or the CO<sub>2</sub> level substantially enhanced the Micronaire, UHM, UI, and bundle strength of parental C312. Therefore, high fiber quality in SPS transgenic plants is more independent of environment.

**Example 4- Summary of Results Demonstrating Increased Fiber Quality as Analyzed by Mantis Single Fiber Tests**

Cotton fibers with higher individual fiber strength are highly valued by the textile industry because they break less frequently during processing. Therefore, average fiber length can be maintained at a higher value throughout processing and higher quality fabrics can be manufactured with fewer defects. Increasing individual fiber strength is a major goal of the cotton industry.

Mantis tests to determine single fiber strength were run on 100 fibers (two independent groups of 50 fibers each) from at least 4 plants from each plant line. Therefore, data in Table 5 are averages from at least 400 total fibers from each plant line.

**Table 5**

**Single Fiber Strength of SPS Transgenic Plants Compared to Parental C312 (at 30/15°C and 360 ppm CO<sub>2</sub>)**

Plant Line	Fiber #	Tb (g)	Normalized Tb	Tb S.D.	Tb S.D. %	Elong (%)	Change in Elong %	Work (μJ)	Normalized Work	Work S.D.	Work S.D. %
C312-wt	400	5.30	1.00	2.45	46.2	15.05		13.21	1.00	8.98	68.0
13-3a											
T1#1@T2	400	5.90	1.11	2.55	43.2	17.40	+2.35	15.99	1.21	8.62	53.9
225-17a											
T1	400	7.18	1.35	2.85	39.7	16.67	+1.62	18.09	1.37	9.55	52.8
40-4b											
T1#1,#4@T2	500	6.60	1.24	2.71	41.1	16.89	+1.84	17.22	1.30	9.21	53.5
Transgenic Average		6.56	1.24	2.70	41.2	16.99	+1.94	17.10	1.29	9.13	53.4

- 20 Tb: grams of force to break a single fiber  
 Elong %: single fiber elongation before break as % of original length  
 Work: a composite of Tb and Elongation = work expended to break a single fiber  
 XX S.D: Standard deviation of the value  
 XX S.D. %: % of the actual value represented by the standard deviation value

25

Table 5 shows that single fiber strength as manifested in Tb, Elongation, and Work is consistently improved in all 3 transgenic lines compared to parental C312. On average in three transgenic lines, Tb is increased 24% to 6.56 g (Fig. 12), Elongation is increased 1.94% to 16.99% (Fig. 13), and Work is increased 29% to 17.10 μJ (Fig. 14).

(HVI did not show any increase in Elongation % of transgenic lines compared to parental C312 because the bundle-based HVI test will reflect only the elongation of the weakest fibers in the bundle.) Also, the standard deviation is a lower percentage of the transgenic single fiber strength values (averaging 14.6% lower for Work), demonstrating improved  
 5 uniformity of single fiber strength. (Results of Mantis single fiber tests are expected to have high standard deviations).

The scatter plots in Figs. 15 – 19 show correlations between single fiber strength parameters and Micronaire or Fiber Weight per Seed from the 30/15°C, 360 ppm CO<sub>2</sub> chamber. These illustrate positive correlations between Tb and Work and Micronaire and  
 10 Fiber Weight per Seed (Figs. 15-18). In contrast, no positive correlations were observed between Elongation and Micronaire (Fig. 19) or Fiber Weight per Seed. Coefficients of determination show that 39 - 68% of the increases in Tb and Work are determined by increases in Micronaire and Fiber Weight per Seed. These positive correlations are primarily determined by distinctly separated groups of data points from the fibers of SPS  
 15 transgenic plants. This point is emphasized by Table 6 showing coefficients of determination ( $R^2$ ) for each plant line considered separately. In contrast to the transgenic lines, parental C312 shows no substantial, positive  $R^2$  values. Therefore, over-expression of SPS causes increased values of Micronaire in transgenic fibers that are correlated with increased values of single fiber strength compared to parental C312.

20

**Table 6**  
**Coefficients of Determination ( $R^2$ ) from Linear Regression Plots**  
**of Single Fiber Strength Parameters of Individual Plant Lines Plotted Against**  
**Micronaire and Fiber Weight Per Seed**

Y Axis	Work		Tb		Elongation	
X Axis	Micronaire	Fiber Weight per Seed	Micronaire	Fiber Weight per Seed	Micronaire	Fiber Weight per Seed
Plant Line						
C312	-0.10	-0.10	0.16	0.15	-0.29	-0.29
13-3a	0.50	0.06	0.37	0.00	0.56	0.30
225-17a	0.40	0.67	0.95	0.99	-0.57	-0.31
40-4b	0.34	0.83	0.83	0.54	0.10	0.83

25

The substantial positive correlations with Tb and Work for both Micronaire (in 3 transgenic lines) and Fiber Weight per Seed (in 2 transgenic lines) support the fact that the increases in Fiber Weight per Seed and Micronaire are due to increased cellulose



deposition in the fiber wall. Increase in Fiber Weight per Seed due to increased fiber number or increase in Micronaire due to increased fiber diameter would not result in an increase in single fiber strength. (Note that fiber number per seed cannot be determined, whereas the data allow one to predict by standard methods that fiber diameter has not changed.) However, the lack of complete correlation between single fiber strength values and Micronaire and Fiber Weight per Seed suggests that over-expression of SPS also contributes independently to increased single fiber strength, with 52 - 61% of the increased work values being explained by factors other than increased wall thickness. Also, the tendency for elevated Elongation in transgenic fibers is, as expected, independent of increased cellulose content of the fiber wall. (Elongation is highly dependent on the orientation of cellulose microfibrils within the fiber wall.) This point is emphasized by comparing line 13-3a with other transgenic lines.

#### **Example 5 - Photosynthetic Efficiency Under Cool Night Temperatures**

Over-expression of SPS in the leaves increases tolerance to cool nights by maintaining photosynthetic rates equal to warm-grown plants during the warm days following a 15°C night. In contrast, untransformed cotton shows reduced photosynthetic rate in the warm day following a cool night.

Transgenic plants and parental C312 plants growing in the Phytotron were assayed for photosynthetic efficiency between 7 - 14 weeks of age. The first fully expanded leaf from the apex (judged by dark green color, shape, and size--the 3rd or 4th leaf down) was clamped and assayed for photosynthetic efficiency using a ADC LCA-4 analyzer under variable internal CO<sub>2</sub> concentrations. Plants growing at 30/28°C were assayed between 7 - 10 weeks of age and plants growing at 30/15°C were assayed between 10 - 14 weeks of age. In the earliest case, the plants would have been exposed to the experimental conditions for about 4 weeks. The plants were assayed at 30°C and at 4 h into the photoperiod, which also represented 3 h after complete rewarming from 28°C or 15°C to 30°C. Two plants were assayed for each line in each chamber.

The graphs show photosynthetic rates over a range of internal CO<sub>2</sub> concentrations for parental C312 (Fig. 21) and two transgenic lines, 13-3a-1 (Fig. 22) and 225-17a (Fig. 23). Normal atmospheric CO<sub>2</sub> concentration corresponds to internal CO<sub>2</sub> concentration of about 270  $\mu\text{L L}^{-1}$ . Each graph is a compilation of four scatter plots, one for each plant of the line that was tested. The relative placement of empty symbols

(30/15°C condition) and filled symbols (30/28°C condition) should be compared between the lines. Comparing photosynthetic rate below internal CO<sub>2</sub> concentrations of 500 µL L<sup>-1</sup>, all four plants in the two transgenic lines tested maintained, when growing under a 30/15°C cycle, the same photosynthetic rate during the warm day as was observed for plants growing under 30/28°C cycling. In contrast, parental C312 showed the expected cool-night-induced reduction in photosynthetic rate, even though the assay was always done during the warm day. For three of the four transgenic plants tested, this difference was maintained at all internal CO<sub>2</sub> concentrations tested.

The variability in plant age at the time of assay between 30/15°C and 30/28°C chambers means that the comparisons between temperature cycles should be considered tentative. However, use of the same type of leaf from actively growing plants in each case supports their usefulness.

It is not yet known why plants over-expressing SPS fail to acclimate photosynthesis in response to chilling as occurs in parental C312. Future analyses of leaf carbohydrate content will indicate whether more sucrose is synthesized during the warm day in transgenic plant leaves, which, coupled with higher rates of photosynthesis, might result in greater carbohydrate export from leaves to developing fibers during the day than occurs in parental C312. Such a mechanism could contribute to the increased seed and fiber yield and fiber quality of plants over-expressing SPS. It has also been observed that transgenic plants over-expressing SPS store less starch in their hypocotyls than parental C312. This indicates another source of extra carbohydrate that could help increase seed and fiber yield and fiber quality.

#### **Example 6 - Shift of Metabolic Flux Toward Cellulose in Sink Cells**

Tables 2 and 3 show that fiber properties depending on cellulose content, including fiber weight/seed, micronaire, and fiber maturity ratio, increase in transgenic plants when SPS activity is elevated both in the leaves and the fibers. Therefore, with whole-plant analyses, one cannot judge whether these improvements are aided by enhanced export of sucrose from the leaves to the fibers or enhanced synthesis of sucrose in fiber (sink) cells, or both. Since cellulose synthesis has been proposed to use sucrose as an obligatory substrate from which UDP-glucose is generated by the enzyme sucrose synthase, SPS within sink cells can promote metabolic flux toward cellulose by one or both of two mechanisms. SPS could resynthesize sucrose within sink cells because

translocated sucrose is cleaved before or soon after entering them, and/or SPS could reuse the fructose released by the activity of sucrose synthase to synthesize more sucrose (Fig. 2).

Evidence that metabolic flux toward cellulose synthesis is enhanced in cellulose-storing sink cells (represented by cotton fibers) by over-expression of SPS was obtained from cotton ovules with attached developing fibers cultured *in vitro*. Cultured ovules/fibers are a non-photosynthetic system that uses external glucose in plant tissue culture medium as a carbon source to support metabolism required for seed and fiber maturation. Accepting that sucrose is an obligatory substrate for fiber cellulose synthesis, SPS synthesizes sucrose within tissue-cultured ovules/fibers supplied only with glucose. SPS could also reuse the fructose released by the activity of sucrose synthase to synthesize more sucrose. Positive effects of SPS over-expression observed in this system are necessarily independent of photosynthesis. However, the substrate supply in this tissue culture system is constant, implying that it is not possible to exclude enhanced supply of sucrose due to enhanced SPS expression in leaves or decreased starch storage in hypocotyls as also important in improvements observed in whole plants

Plants yielding the results in Table 7 were flowering in the greenhouse between July and December. Ovules were dissected from flowers and cultured at 34°C on 1 DPA. The ovules of one flower were split between the 34°C and 15°C comparison in each case. Comparison within one flower better controlled the variability that was observed in the rates of cellulose synthesis on 21 DPA between cultures from different flowers of the same plant line. Each test at each temperature included 12 – 18 ovules split between three replicate dishes. Cultures were shifted from constant 34°C to a 34/15°C 12h/12h cycle on 18 DPA when secondary wall deposition had commenced. <sup>14</sup>C-glucose was used to label developing ovules and fibers on 21 DPA at 34°C and 15°C. Therefore, the cultures had 3 days to adjust to exposure to 15°C, and on 21 DPA the 15°C assay was run 4 h after the shift to 15°C. Cultures of parental C312 treated identically were almost always assayed in parallel with transgenic plant lines.

Rates of respiration (<sup>14</sup>CO<sub>2</sub> evolution) and rates of crystalline cellulose synthesis (<sup>14</sup>C-cellulose remaining insoluble after boiling in acetic/nitric reagent) were determined at both temperatures. Metabolic activity of ovules (seeds) and cotton fibers is combined in the resulting data. However, previous work in which ovules and fibers were separated

after the assay was completed demonstrated that under 34/15°C conditions, 82% of the total cellulose dpm (in ovules + fibers) was attributable to the fibers alone.

From the  $^{14}\text{CO}_2$  and  $^{14}\text{C}$ -cellulose data, four values were calculated for each plant line: (1) R% - a percentage derived from the 15°C/34°C ratio of dpm  $^{14}\text{CO}_2$  trapped on a KOH-soaked filter paper in the incubation chamber; (2) C% - a percentage derived from the 15°C/34°C ratio of dpm  $^{14}\text{C}$ -cellulose remaining insoluble after boiling in acetic/nitric reagent; (3) C/R<sub>15</sub> - the ratio between dpm  $^{14}\text{C}$ -cellulose and dpm  $^{14}\text{CO}_2$  at 15°C; and (4) C/R<sub>34</sub> - the ratio between dpm  $^{14}\text{C}$ -cellulose and dpm  $^{14}\text{CO}_2$  at 34°C. R% and C% describe the proportion of the 34°C rate of respiration or cellulose synthesis, respectively, that can be maintained at 15°C. C/R<sub>15</sub> and C/R<sub>34</sub> describe the proportion of metabolic flux directed toward cellulose synthesis vs. respiration at 15°C or 34°C, respectively. Results from parental C312 and 7 transgenic lines tested with good replication in parallel are shown in Table 7 with values considered higher than parental C312 shown in bold.

Table 7

**Data Calculated From Rates of Cellulose Synthesis and Respiration at 34°C and 15°C in *in vitro* Cultures**

Plant Line	Number of Tests	R%	C%	C/R <sub>34</sub>	C/R <sub>15</sub>
C312-wt	12	17.2	21.5	2.8	3.5
13-3a*	6@T2	15.3	21.8	1.8	3.0
38-4a	7@T2	13.0	<b>25.7</b>	1.9	<b>3.9</b>
40-4b*	5@T2	13.1	<b>25.4</b>	1.9	<b>3.7</b>
40-6a*	6@T2	15.4	20.4	2.8	<b>3.7</b>
58-3a	4@T1	14.3	<b>25.9</b>	3.4	<b>6.2</b>
225-17a*	4@T1	<b>20.9</b>	<b>22.6</b>	2.8	3.1
619-1a	7@T1	15.9	<b>24.9</b>	2.9	<b>4.6</b>

\* indicates lines shown in the Phytotron to have improved fiber quality.

The data in Table 7 show that over-expression of SPS reduces R% in 6 of 7 transgenic lines tested in parallel compared to parental C312. This is paralleled by an increase in C% in 5 of 7 transgenic lines tested, meaning that most SPS transgenic lines

are able to synthesize cellulose more efficiently at 15°C than parental C312.

Correspondingly, the ratio of cellulose synthesis rate to respiration rate at 15°C ( $C/R_{15}$ ) increases in 5 of 7 transgenic lines tested. One transgenic line showed an increase in  $C/R_{34}$ . Transgenic line 13-3a that showed improved fiber quality in the Phytotron did not show improvement in this assay except for reduction of R%. Perhaps this is because secondary wall production proceeds less vigorously *in vitro* than *in planta*.

**Example 7 - Higher Rate of Weight Gain in Sink Cells (Cotton Fibers) During Primary and Secondary Wall Deposition**

The *in vitro* ovule/fiber culture system has provided direct evidence that over-expression of SPS in sink cells can lead to higher rates of fiber weight gain at both warm and cool temperatures by mechanisms independent of photosynthesis.

Ovules of transgenic and control C312 were cultured *in vitro* at constant 34°C or cycling 34/15°C from the beginning of culture. Ovules/fibers (8-10 per data point) were harvested from parallel cultures (containing equal representation of 5-8 flowers from at least 3 plants) at intervals during fiber maturation (12 - 45 DPA). Fibers were stripped from ovules, oven-dried, and weighed. Fiber weight was plotted against time and the slope of weight gain during the period of high-rate secondary wall cellulose synthesis was determined under both temperature regimes. A ratio for the 34/15°C:34°C slopes within one plant line was also calculated, which will normalize for any inherent differences in rates of fiber weight gain in cultures of particular lines. For most plant lines tested, several replications of the experiment were conducted at various times allowing average slopes to be compared. A second experiment during a second compressed time interval included 3 complete time-course replications of fiber weight gain in the transgenic plant lines grown in the Phytotron, plus line 38-4a-1. The results of this second experiment, which indicate the repeatability of this assay, are shown as separate italic entries in the table. Values substantially greater than are found in the C312 parental line are highlighted in bold in Table 8.

Table 8

Rates of Cellulose Deposition in Fibers Cultured *in vitro* at 34°C or 34/15°C

Plant Line	34°C slope	34/15°C slope	Ratio 34/15°C:34°C slope
C312-wt	0.54	0.33	0.61
C312-wt	0.52	0.31	0.60
13-3a-1*	0.37	0.31	0.84
13-3a-1*	0.45	0.39	0.87
38-4a-1	0.45	0.25	0.56
40-4b-1*	0.55	0.19	0.34
40-4b-1*	0.46	0.24	0.52
-2	0.36	0.25	0.69
-2KS**	0.38	0.26	0.68
40-6a-1	0.38	0.30	0.78
-4*	0.22	0.10	0.45
40-17a-6	0.34	0.28	0.82
58-3a	0.42	0.41	0.98
178-1a	0.49	0.20	0.41
225-17a*	0.46	0.24	0.52
225-17a*	0.58	0.26	0.45
414-1a	0.63	0.39	0.62
619-1a	0.60	0.37	0.62

5           \*Tested at the Phytotron; showing improved fiber quality.

KS\*\*; A kanamycin-sensitive sibling of the kanamycin-resistant plant described immediately above; the kanamycin-sensitive sibling from a population of segregating seeds is expected not to carry a copy of the foreign genes. Note that the slopes from the kanamycin-sensitive and kanamycin-resistant siblings of 40-4b-2 are almost identical, and  
 10   the differences between these and slopes from the parental C312 cannot be related to expression of the foreign gene.

Line 40-6a and 40-17a are listed together and counted as one line because they likely represent the same transformation event based on derivation from the same parent callus and the same segregation ratio at T1.

Two of the transgenic lines (414-1a and 619-1a) had rates of fiber weight gain at 34°C higher than parental C312, and several more had higher rates than the non-SPS-expressing transgenic line, 40-4b-2-KS. Four transgenic lines (13-3a, 58-3a, 414-1a, and 619-1a) had rates of fiber weight gain at 34/15°C higher than parental C312. Three  
 5 transgenic lines (13-3a-1, 40-6a-1 = 40-17a-6, 58-3a) had a ratio for the 34/15°C:34°C slopes higher than parental C312 and the non-SPS-expressing transgenic line, 40-4b-2-KS. Lines 414-1a and 619-1a do not stand out in analysis of slope ratios because of greater slopes at both 34°C and 34/15°C, but these are promising lines for future fiber quality analysis. Some of the lines tested at the Phytotron and shown to have improved  
 10 fiber quality are superior to parental C312 in this test. The lack of complete consistency may be due to the fact that secondary wall production proceeds less vigorously *in vitro* than *in planta*.

From replicated time-courses of fiber weight gain, absolute values of fiber dry weight were also compared at 15 DPA (end of primary wall deposition) and 30 DPA  
 15 (after extensive secondary wall deposition) in the transgenic plant lines grown in the Phytotron, plus line 38-4a-1. Each data point is the average from three experiments, including fiber from a total of 24 – 30 ovules representing 15 – 24 flowers from 4 – 6 plants per line. The results are shown in Table 9.

20

Table 9

Weights of Fiber (mg/ovule) from *in vitro* Cultures

Plant Line	15 DPA			30 DPA		
	34°C	34/15°C	Ratio 34/15°C:34°C weights	34°C	34/15°C	Ratio 34/15°C:34°C weights
C312-wt	1.75	0.46	0.263	8.89	3.88	0.436
13-3a-1*	1.94	0.60	0.309	7.33	4.64	0.633
38-4a-1	1.68	0.67	0.399	8.68	3.68	0.424
40-4b-1*	2.18	0.64	0.294	7.36	3.48	0.473
225-17a*	1.84	0.59	0.320	8.80	3.72	0.423

25

\*Tested at the Phytotron; showing improved fiber quality.

At 15 DPA, four transgenic lines show consistently greater weight gain than parental C312 under 34/15°C, and three of the four transgenic lines show greater weight gain under constant 34°C. The ratio of 34/15°C to 34°C weights is greater in all four transgenic lines, demonstrating improved fiber production in SPS transgenic plants under  
5 adverse cool temperatures by mechanisms independent of photosynthesis. At 15 DPA, fiber dry weight is composed mostly of primary walls, and greater fiber weight could be due to greater fiber length or greater primary wall thickness, or both.

At 30 DPA, one transgenic line shows greater fiber weight gain than parental C312 under 34/15°C. Two transgenic lines show greater ratio of 34/15°C to 34°C  
10 weights. Fiber dry weight at 30 DPA is largely cellulose. Therefore, SPS over-expression within transgenic fibers promotes cellulose deposition, including its deposition under adverse cool temperatures. The inconsistency of results for transgenic lines at 30 DPA is likely explained by the fact that secondary wall deposition *in vitro* is more hindered than fiber lengthening. However, all the transgenic lines tested in the Phytotron  
15 and showing improved fiber quality show some improvement in this *in vitro* test.

#### **Example 8 – Enhanced Stem Weight of Transgenic Cotton Plants**

The positive effects of SPS over-expression on cellulose synthesis in cotton fibers  
20 extends to other fibers. Fibers make up most of the weight of annual or perennial strong stems, such as are found in mature cotton plants. Therefore, the stem weight of cotton plants grown in the Phytotron and the Texas Tech greenhouse was determined (Table 10). The conditions of the Texas Tech greenhouse were most similar to the Phytotron 30/15°C, 360 ppm CO<sub>2</sub> chamber.



**Table 10****Normalized Values for Stem Weight, Diameter, and Height**

(Average values for transgenic plants are normalized to the corresponding value for the Coker 312 wild-type parent set to 1.00.)

5

Plant Line	Phytotron Test					Greenhouse Test			
	Phytotron Plants (n) per chamber, in order	Stem Weight 30/15°C CO <sub>2</sub> =360	Stem Weight 30/15°C CO <sub>2</sub> =700	Stem Weight 30/28°C CO <sub>2</sub> =360	Stem Weight 30/28°C CO <sub>2</sub> =700	Green House Plants (n)	Stem Weight	Stem Diameter	Stem Height
C312-wt	4,4,4,4	1.00	1.00	1.00	1.00	6	1.00	1.00	1.00
13-3a									
T1#1@T2	4,4,4,4	1.12	1.20	1.03	1.11				
225-17a									
T1	4,4,4,4	0.95	1.11	1.28	1.07				
40-4b									
T1#1&#4@T2	5,5,7,5	0.81	1.12	1.22	1.13				
40-6a									
T1#4@T2	1,1,2,0	1.33	1.30	1.82	—				
T2-4-3@T3						5	1.27	1.11	1.06
357-6a									
T1#1@T2						6	0.92	0.93	0.94

In the Phytotron, time of stem weight determination varied somewhat between plant lines for the 30/28°C chambers because each plant was harvested shortly after all  
 10 bolls on it had opened. For the 30/15°C condition, plant growth was terminated at the same time when some immature bolls remained on all plants. All plants were 6- 7 months old at time of harvest. In the Texas Tech greenhouse, parental and transgenic plants were randomized on two adjacent tables and grown for 30 weeks before simultaneous harvesting. Main stem diameter and height were also determined in the  
 15 greenhouse plants.

In the Phytotron, stem weight increased by 10% or more in transgenic plants compared to parental C312 in 11 of 15 cases (representing the matrix of plant lines x chambers tested). The increases are particularly pronounced and consistent across three chambers for line 40-6a-4, although there were few replicate plants in the Phytotron for this line. Therefore, line 40-6a-4-3 was tested at the next generation (T3) in the Texas  
 20 Tech greenhouse with more replication in parallel with parental C312 and another transgenic line, 357-6a-1 at T2. Line 40-6a-4-3 again showed average increased stem weight with a similar magnitude of change as observed in the Phytotron chambers at 30/15°C and both 360 and 700 ppm CO<sub>2</sub>. In addition, line 40-6a-4-3 showed average

increased stem height and stem diameter compared to parental C312 and the transgenic line 357-6a-1, which was smaller than C312. Therefore, transgenic lines do not all show increased stem weight, probably because of differences in tissue-specific gene expression. Considering the main plant stem, excluding branches that were also weighed, as a right cone with volume =  $\pi r^2 h / 3$ , line 40-6a-4-3 would have increased volume of 1.31 times compared to parental C312. The similarity of this to the observed weight increase of 1.27 times suggests that much of the weight increase is associated with increased volume of the main stem containing abundant fibers. The 4% difference between the theoretical prediction and the observation could be due to different degrees of branching or changes in stem density that have not been determined.

#### **Example 9 - Increased Stem Diameter in Multiple Lines of Transgenic Cotton**

In addition to line 40-6a, some stems appeared bigger than others among transgenic cotton plants growing in the greenhouse. However, these plants were of different ages. To try to quantitate this observation, electronic calipers were used to measure stem diameter approximately two inches above the soil line in all plants in the greenhouse on 9/23/98 (which did not include all the plants of interest implicated by previous studies). Date of planting was also recorded for each plant measured. By analyzing values for the Coker 312 parent and transgenic line 58-3a(2) (T1 individuals, number 1 -7) that had plants of several ages in the greenhouse, the following approximate values for rate of stem diameter increase per day were estimated. The rate decreases with time because, in the 2 gallon pots used for planting, stem diameter in parental C312 plants apparently slows or stops increasing at about 5 months.

<u>Plant Age</u>	<u>Rate of Stem Diameter Increase</u>
< 150 days	0.13 mm/day
160 - 200 days	0.10 mm/day
>210 days	0.06 mm/day

Of 12 independent transgenic lines analyzed (each with several replicate pots), six had average values greater than the standards established for parental C312 (or at the upper end of the range) (Table 11). Transgenic lines that did not show increased rates of stem diameter increase may express spinach SPS less strongly in their stems.

**Table 11**  
**Transgenic Plant Lines with Enhanced Rates of**  
**Stem Diameter Increase in the Greenhouse**

Plant Line	Plant Age (days)	Rate of Stem Diameter Increase (mm/day)
40-4b-2-7	216	0.076
40-6a-4-2	180	0.124
-3,4	215	0.107
58-3a-3	214	0.078
414-1a-1,2	193	0.086
530-1a-2,3	197	0.095
619-1a-6	153	0.140

Note that Table 10 confirms through a second experiment the increased rate of stem diameter increase for line 40-6a-4-3. Increased stem diameter depends on more cellulose-containing fiber within the stem. Larger stem diameter at the end of a growing period could be explained by faster rate of diameter increase or longer persistence of diameter increase in one growing season. Either case will result in more harvestable stem fiber.

**Example 10 - Enhanced Conversion of Atmospheric CO<sub>2</sub> into Harvestable Crops, Preferentially Cellulose-based Fiber**

As shown in Table 12, comparison of data between the 30/15°C Phytotron chambers with 360 and 700 ppm CO<sub>2</sub> demonstrates that SPS transgenic plants convert normal levels of CO<sub>2</sub> more efficiently into cellulose-based cotton fiber. At normal levels of CO<sub>2</sub>, SPS transgenic plants are able to more nearly reach their maximum possible fiber production potential (as shown by comparative changes in Lint Fiber Weight per Seed) so that raising CO<sub>2</sub> to 700 ppm increases their fiber wall thickness less than parental C312 (as shown by comparative changes in Micronaire). However, when stem weight is considered as an indication of production potential for all types of fiber, transgenic plants remain superior to parental C312 at 30/15°C even under elevated CO<sub>2</sub>. In contrast, raising CO<sub>2</sub> levels at 30/15°C tended to decrease seed weight in transgenics and parental

C312 (although transgenic seed weight always remained higher than in parental C312—see Example 2).

Therefore, over-expression of SPS has a preferential effect on cotton fiber production probably due to increasing sink demand of this cellulose-based sink. SPS over-expression in fiber can, as previously demonstrated, preferentially increase metabolic flux toward cellulose and fiber weight gain. Data supporting these conclusions are shown in Table 12, which shows the percentage change in values of various parameters when CO<sub>2</sub> was increased from 300 to 700 ppm under 30/15°C in the Phytotron.

**Table 12**

**Percentage Change in Various Crop-Related Attributes  
With Increase from 300 to 700 ppm CO<sub>2</sub> at 30/15°C**

Plant Line	Micro- naire	Lint Fiber Weight per Seed	Fuzzy Seed Weight per Seed	Ratio of Fiber to Fuzzy Seed Weight	Stem Weight
C312-wt	+9%	+35%	-8%	+48%	+22%
13-3a-1@T2	+2%	+10%	-6%	+18%	+31%
225-17a@T1	-18%	-5%	-14%	+12%	+42%
40-4b-1,4@T2	+7%	+25%	0%	+24%	+71%
Transgenic Average	-3%	+10%	-7%	+18%	+48%

Fiber crops that over-express SPS can convert normal CO<sub>2</sub> more efficiently into economically valuable fiber. Such plants grown widely as crops should help to combat rising CO<sub>2</sub> levels in the atmosphere because they immobilize CO<sub>2</sub> into fiber cellulose with improved efficiency under normal CO<sub>2</sub> levels, and this efficiency of production is maintained (for cotton fiber) or enhanced (for stem fiber) under elevated CO<sub>2</sub> levels.

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

What is claimed:

1. A transgenic cotton plant wherein the transgenic cotton plant has an increased level of sucrose phosphate synthase relative to a non-transgenic cotton plant.  
5
2. The transgenic cotton plant according to claim 1, wherein the cotton plant is transformed with a chimeric DNA construct that expresses sucrose phosphate synthase.
3. The transgenic cotton plant according to claim 1, wherein the chimeric  
10 DNA construct comprises a plant specific promoter.
4. The transgenic cotton plant according to claim 1, wherein the chimeric DNA construct is stably integrated into the genome of the cotton plant.
- 15 5. The transgenic cotton plant according to claim 1, wherein the chimeric DNA construct is introduced into the cotton plant by a method selected from the group consisting of electroporation, *Agrobacterium* mediated transformation, biolistic gene transformation, chemically mediated transformation, and microinjection.
- 20 6. The transgenic cotton plant according to claim 1, wherein the sucrose phosphate synthase is selected from the group consisting of spinach, *Arabidopsis*, beet, bean, citrus, maize, moss, potato, rice, sugar cane, and *Synechocystis* sucrose phosphate synthase.
- 25 7. The transgenic cotton plant according to claim 6, wherein the sucrose phosphate synthase is spinach sucrose phosphate synthase.
8. The transgenic cotton plant according to claim 1, wherein cotton fibers from the plant have improved quality.
- 30 9. The transgenic cotton plant according to claim 1, wherein cotton fibers from the plant have an improved quality selected from the group consisting of increased

strength, increased length, and increased micronaire, as compared to a cotton plant lacking the transgene.

10. Seed produced from the plant according to claim 1.

5

11. A method of increasing the yield of cotton plant comprising:  
introducing into a cotton plant a chimeric DNA construct capable of altering  
sucrose phosphate synthase activity in an amount sufficient to increase the yield of the  
cotton plant.

10

12. The method according to claim 11, further comprising:  
growing said cotton plant.

13. The method according to claim 11, wherein the yield of cotton seeds is  
15 increased.

14. The method according to claim 11, wherein the yield of cotton fiber is  
increased.

20 15. The method according to claim 11, wherein the chimeric DNA construct  
expresses a sucrose phosphate synthase.

16. The method according to claim 15, wherein the sucrose phosphate  
synthase is selected from the group consisting of spinach, *Arabidopsis*, beet, bean, citrus,  
25 maize, moss, potato, rice, sugar cane, and *Synechocystis* sucrose phosphate synthase.

17. The method according to claim 16, wherein the sucrose phosphate  
synthase is spinach sucrose phosphate synthase.

30 18. The method according to claim 11, wherein the chimeric DNA construct  
comprises a plant specific transcription initiation region.

- 50 -

19. The method according to claim 18, wherein the transcription initiation region is tissue specific.

20. The method according to claim 18, wherein the transcription initiation  
5 region is leaf specific.

21. The method according to claim 18, wherein the transcription initiation region is a RUBISCO small subunit promoter, a 35S promoter, a fiber enhanced promoter, a vascular cell enhanced promoter, a stem cell enhanced promoter, or a seed  
10 enhanced promoter.

22. The method according to claim 15, wherein the chimeric DNA construct is stably integrated into the genome of the cotton plant.

15 23. The method according to claim 15, wherein said introducing of the chimeric DNA construct is into the plant is carried out by a method selected from the group consisting of electroporation, *Agrobacterium* mediated transformation, biolistic gene transformation, chemically mediated transformation, and microinjection.

20 24. A method of increasing the quality of cotton fiber produced from a cotton plant comprising:  
introducing into a cotton plant a chimeric DNA construct capable of altering sucrose phosphate synthase activity in an amount sufficient to increase the quality of the cotton fiber produced by the cotton plant.

25 25. The method according to claim 24, further comprising:  
growing said cotton plant.

26. The method according to claim 24, wherein cotton fiber has an improved  
30 quality selected from the group consisting of increased strength, increased length, and increased micronaire, as compared to a cotton plant lacking the transgene.

27. The method according to claim 24, wherein the chimeric DNA construct expresses a sucrose phosphate synthase.

28. The method according to claim 27, wherein the sucrose phosphate  
5 synthetase is selected from the group consisting of spinach, *Arabidopsis*, beet, bean, citrus, maize, moss, potato, rice, sugar cane, and *Synechocystis* sucrose phosphate synthase.

29. The method according to claim 28, wherein the sucrose phosphate  
10 synthase is spinach sucrose phosphate synthase.

30. The method according to claim 24, wherein the chimeric DNA construct comprises a plant specific transcription initiation region.

31. The method according to claim 30, wherein the transcription initiation  
15 region is tissue specific.

32. The method according to claim 30, wherein the transcription initiation  
20 region is leaf specific.

33. The method according to claim 30, wherein the transcription initiation  
region is a RUBISCO small subunit promoter, a 35S promoter, a fiber enhanced  
promoter, a vascular cell enhanced promoter, a stem cell enhanced promoter, or a seed  
enhanced promoter.

34. The method according to claim 24, wherein the chimeric DNA construct is  
25 stably integrated into the genome of the cotton plant.

35. The method according to claim 24, wherein said introducing of the  
30 chimeric DNA construct into the plant is carried out by a method selected from the group consisting of electroporation, *Agrobacterium* mediated transformation, biolistic gene transformation, chemically mediated transformation, and microinjection.



36. A method of regulating the ratio of cellulose to other dry weight components of a plant, comprising:

introducing into a plant a chimeric DNA construct capable of altering sucrose phosphate synthase activity in an amount sufficient to regulate the ratio of cellulose to  
5 other dry weight components of the plant.

37. The method according to claim 36, further comprising:  
growing said plant.

10 38. The method according to claim 36, wherein the ratio of cellulose to other dry weight components of a plant is increased.

39. The method according to claim 36, wherein the chimeric DNA construct expresses a sucrose phosphate synthase.

15

40. The method according to claim 39, wherein the sucrose phosphate synthase is selected from the group consisting of spinach, *Arabidopsis*, beet, bean, citrus, maize, moss, potato, rice, sugar cane, and *Synechocystis* sucrose phosphate synthase.

20 41. The method according to claim 40, wherein the sucrose phosphate synthase is spinach sucrose phosphate synthase.

42. The method according to claim 36, wherein the chimeric DNA construct comprises a plant specific transcription initiation region.

25

43. The method according to claim 42, wherein the transcription initiation region is tissue specific.

44. The method according to claim 42, wherein the transcription initiation  
30 region is leaf specific.

45. The method according to claim 42, wherein the transcription initiation region is a RUBISCO small subunit promoter, a 35S promoter, a fiber enhanced

promoter, a vascular cell enhanced promoter, a stem cell enhanced promoter, or a seed enhanced promoter.

46. The method according to claim 36, wherein the chimeric DNA construct is  
5 stably integrated into the genome of the plant.

47. The method according to claim 36, wherein said introducing of the  
chimeric DNA construct into the plant is carried out by a method selected from the group  
consisting of electroporation, *Agrobacterium* mediated transformation, biolistic gene  
10 transformation, chemically mediated transformation, and microinjection.

48. The method according to claim 36, wherein the ratio of cellulose in dry  
weight components increases to exceed 40%.

49. The method according to claim 48, wherein the increase in cellulose ratio  
15 occurs in xylem cells.

50. The method according to claim 48, wherein the increase in cellulose ratio  
occurs in phloem cells.

20

51. The method according to claim 36, wherein the plant is selected from the  
group consisting of sugarcane, sugar beets, forest trees, forage crops, fiber producing  
plants, and seed producing plants.

52. A method of increasing tolerance of photosynthetic efficiency to cool night  
25 temperatures, comprising:

introducing into a plant a chimeric DNA construct capable of altering sucrose  
phosphate synthase activity in an amount sufficient to increase tolerance of  
photosynthetic efficiency to cool night temperatures.

30

53. The method according to claim 52, further comprising:  
growing said plant.

- 54 -

54. The method according to claim 53, wherein the chimeric DNA construct expresses a sucrose phosphate synthase.

55. The method according to claim 54, wherein the sucrose phosphate  
5 synthetase is selected from the group consisting of spinach, *Arabidopsis*, beet, bean, citrus, maize, moss, potato, rice, sugar cane, and *Synechocystis* sucrose phosphate synthase.

56. The method according to claim 55, wherein the sucrose phosphate  
10 synthase is spinach sucrose phosphate synthase.

57. The method according to claim 52, wherein the chimeric DNA construct comprises a plant specific transcription initiation region.

58. The method according to claim 57, wherein the transcription initiation  
15 region is tissue specific.

59. The method according to claim 57, wherein the transcription initiation  
20 region is leaf specific.

60. The method according to claim 57, wherein the transcription initiation  
region is a RUBISCO small subunit promoter, a 35S promoter, a fiber enhanced  
promoter, a vascular cell enhanced promoter, a stem cell enhanced promoter, or a seed  
enhanced promoter.

61. The method according to claim 52, wherein the chimeric DNA construct is  
25 stably integrated into the genome of the plant.

62. The method according to claim 52, wherein said introducing of the  
30 chimeric DNA construct into the plant is carried out by a method selected from the group consisting of electroporation, *Agrobacterium* mediated transformation, biolistic gene transformation, chemically mediated transformation, and microinjection.

63. A method of regulating the thickness of cell walls in a plant, comprising:  
introducing into a plant a chimeric DNA construct capable of altering sucrose  
phosphate synthase activity in an amount sufficient to regulate the thickness of cell walls  
in a plant.

5

64. The method according to claim 62, further comprising:  
growing said plant.

65. The method according to claim 62, wherein the plant is a fiber producing  
10 plant.

66. The method according to claim 62, wherein the plant is selected from the  
group consisting of sugarcane, sugar beets, forest trees, forage crops, fiber producing  
plants, and seed producing plants.

15

67. The method according to claim 62, wherein the chimeric DNA construct  
expresses a sucrose phosphate synthase.

68. The method according to claim 67, wherein the sucrose phosphate  
20 synthase is selected from the group consisting of spinach, *Arabidopsis*, beet, bean, citrus,  
maize, moss, potato, rice, sugar cane, and *Synechocystis* sucrose phosphate synthase.

69. The method according to claim 68, wherein the sucrose phosphate  
synthetase is spinach sucrose phosphate synthetase.

25

70. A method of increasing the harvestable yield of fiber from a fiber  
containing plant, comprising:  
introducing into a plant a chimeric DNA construct capable of altering  
sucrose phosphate synthase activity in an amount sufficient to increase the harvestable  
30 yield of fiber from a fiber containing plant.

71. The method according to claim 70, further comprising:  
growing said plant.

72. The method according to claim 70, wherein the chimeric DNA construct expresses a sucrose phosphate synthase.

5 73. The method according to claim 72, wherein the sucrose phosphate synthase is selected from the group consisting of spinach, *Arabidopsis*, beet, bean, citrus, maize, moss, potato, rice, sugar cane, and *Synechocystis* sucrose phosphate synthase.

74. The method according to claim 73, wherein the sucrose phosphate  
10 synthase is spinach sucrose phosphate synthase.

75. A method of increasing the harvestable yield of seed from a plant, comprising:  
introducing into a plant a chimeric DNA construct capable of altering  
15 sucrose phosphate synthase activity in an amount sufficient to increase the harvestable yield of seed from the plant.

76. The method according to claim 75, further comprising:  
growing said plant.  
20

77. The method according to claim 75, wherein the chimeric DNA construct expresses a sucrose phosphate synthase.

78. The method according to claim 77, wherein the sucrose phosphate  
25 synthase is selected from the group consisting of spinach, *Arabidopsis*, beet, bean, citrus, maize, moss, potato, rice, sugar cane, and *Synechocystis* sucrose phosphate synthase.

79. The method according to claim 78, wherein the sucrose phosphate  
synthase is spinach sucrose phosphate synthase.  
30

80. A method of altering the quality of fiber isolated from a fiber producing plant, comprising:

introducing into a plant a chimeric DNA construct capable of altering sucrose phosphate synthase activity in an amount sufficient to alter the quality of fiber produced from the plant.

5           81.     The method according to claim 80, wherein the fiber has an altered quality selected from the group consisting of increased strength, increased length, and increased weight per unit length, as compared to a plant lacking the transgene.

10           82.     The method according to claim 80, wherein the fiber has an altered quality selected from the group consisting of decreased strength, decreased length, and decreased weight per unit length, as compared to a plant lacking the transgene.

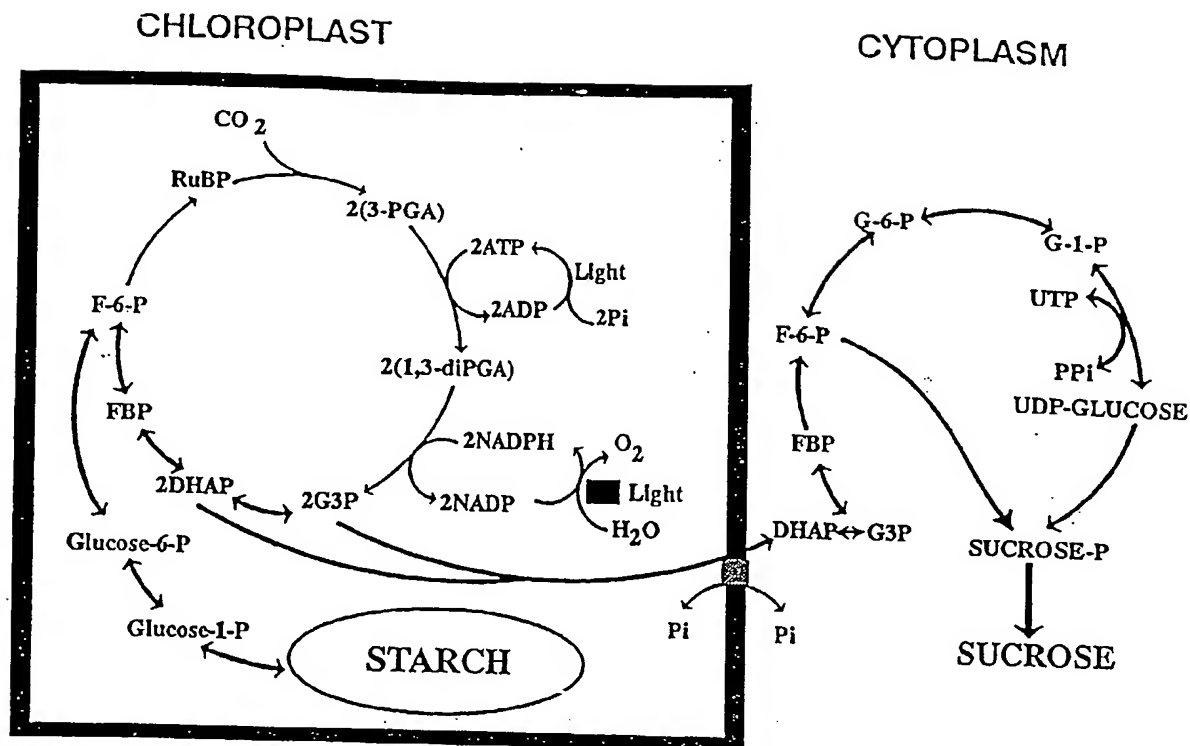


Figure 1

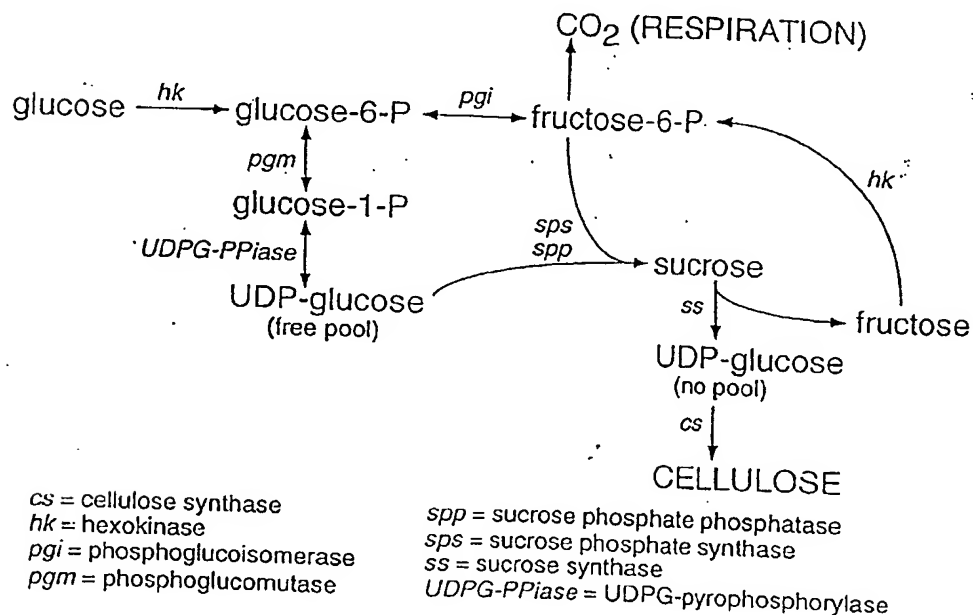


Figure 2

## Plant SPS amino acid alignment

	9	19	29	36	46	56	66	76	86
Spinach SP51									
Citrus unshiu									
C. plantagineum 1									
C. plantagineum 2									
Vicia faba									
S. tuberosum									
Beta vulgaris									
Zea mays									
Oryza sativa 1									
Oryza sativa 2									
A. thaliana 1									
A. thaliana 2									
S. officinarum									
Spinach SP51									
Citrus unshiu									
C. plantagineum 1									
C. plantagineum 2									
Vicia faba									
S. tuberosum									
Beta vulgaris									
Zea mays									
Oryza sativa 1									
Oryza sativa 2									
A. thaliana 1									
A. thaliana 2									
S. officinarum									
Spinach SP51									
Citrus unshiu									
C. plantagineum 1									
C. plantagineum 2									
Vicia faba									
S. tuberosum									
Beta vulgaris									
Zea mays									
Oryza sativa 1									
Oryza sativa 2									
A. thaliana 1									
A. thaliana 2									
S. officinarum									

FIGURE 3



	267	277	284	294	304	314	324	334	344	354	364	
Spinach SP51	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
Citrus unshiu	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
C. plantagineum 1	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
C. plantagineum 2	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
Vicia faba	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
S. tuberosom	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
Beta vulgaris	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
Zea mays	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
Oryza sativa 1	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
Oryza sativa 2	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
A. thaliana 1	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
A. thaliana 2	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
S. officinarum	ESSGAYIIRIPGPKDKYVAKELLP	---	YIPEFVDGALSHIKQSKVLGEQIGGGLPWPASVHGHYADAGDSALLSGALNPMVFTGHSIGRDKLQOLLKQGRLSRE									
	---	---	---	---	---	---	---	---	---	---	---	---
	374	384	394	404	414	424	434	444	454	468		
Spinach SP51	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
Citrus unshiu	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
C. plantagineum 1	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
C. plantagineum 2	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
Vicia faba	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
S. tuberosom	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
Beta vulgaris	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
Zea mays	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
Oryza sativa 1	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
Oryza sativa 2	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
A. thaliana 1	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
A. thaliana 2	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
S. officinarum	EVDATYKIMRRIEAEELCLDASEIVITSTROEIEEQWLYHGFOLVLERKARMRGVSCHGRMPRMVXIIPGMEFNHIAPEADOMDTDIDG----											
	---	---	---	---	---	---	---	---	---	---	---	---
	478	488	498	508	518	528	538	548	558	568	578	
Spinach SP51	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
Citrus unshiu	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
C. plantagineum 1	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
C. plantagineum 2	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
Vicia faba	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
S. tuberosom	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
Beta vulgaris	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
Zea mays	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
Oryza sativa 1	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
Oryza sativa 2	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
A. thaliana 1	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
A. thaliana 2	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
S. officinarum	VINSEIMRFEESNPKPMILALARPDPKKNITTLVKAEGECPRLRELANTLTLINGNRDDIDEMSTSSVLSILIKLIDKDYLGQVAYPKHHKQSDVPDIYRLAARTKGV											
	---	---	---	---	---	---	---	---	---	---	---	---

FIGURE 3 (continued)

Spinach SP51	588	598	608	618	628	638	648	658	668	678	687
Citrus unshiu	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	IGVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
C. plantaginaceum 1	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
C. plantaginaceum 2	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
Vicia faba	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
S. tuberosom	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
Beta vulgaris	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
Zea mays	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
Oryza sativa 1	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
Oryza sativa 2	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
A. thaliana 1	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
A. thaliana 2	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
S. officinarum	FINAFIEPFGTLIEAAAYGLPIVATKNGGVPDI	HRVLDNGLLIDPHDQKSIADALLKLVADKHLMTKCRQNGLNKIHLSFSPHECHKYLSRIASCKPQPNHQRI-DE									
Spinach SP51	696	706	716	726	734	742	752	762	772	782	
Citrus unshiu	GENSDTD-SAGDSLADIQDISLNLKLSLAERTEGNSF--	DDSLDSE--ANAKRTIENAVAKLSK-----									
C. plantaginaceum 1	GGTSES-D-SAGDSLADIQDISLNLKLSLAERTEGNSF--	DDSLDSE--ANAKRTIENAVAKLSK-----									
C. plantaginaceum 2	DDENSED-SAGDSLADIQDISLNLKLSLAERTEGNSF--	DDSLDSE--ANAKRTIENAVAKLSK-----									
Vicia faba	LDATA-IDDSLNDSKDVLDMSRLS--	VGEKMSVNESSESLPGEAAELPDQVRRLNMTKIKRODSQPAQEAEG--									
S. tuberosom	GGSESSES-PGSLADIQDISLNLKLSLAERTEGNSF--	DDSLDSE--ANAKRTIENAVAKLSK-----									
Beta vulgaris	DDENSED-SAGDSLADIQDISLNLKLSLAERTEGNSF--	DDSLDSE--ANAKRTIENAVAKLSK-----									
Zea mays	GLDNQPE-SAGDSLADIQDISLNLKLSLAERTEGNSF--	DDSLDSE--ANAKRTIENAVAKLSK-----									
Oryza sativa 1	ADGABEEFLEDS-MDAQDLSRLS--	TDGEGSSN-----									
Oryza sativa 2	ADGABEEFLEDS-MDAQDLSRLS--	TDGEGSSN-----									
A. thaliana 1	ADGABEEFLEDS-MDAQDLSRLS--	TDGEGSSN-----									
A. thaliana 2	ADGABEEFLEDS-MDAQDLSRLS--	TDGEGSSN-----									
S. officinarum	LDIMKYPEELTS-DSDLDVDDISLRF--	TEGDTLN-----									
Spinach SP51	787	797	806	816	826	836	846	861	871	881	
Citrus unshiu	TSD-----	LLQVKTIVISIGVEQ-RPTGSGIFILSTMTLSEVDSLLDGGGLRPADFAFTCNSSGSELYYSTVSESP-----									
C. plantaginaceum 1	TTG-----	LLQVKTIVISIGVEQ-RPTGSGIFILSTMTLSEVDSLLDGGGLRPADFAFTCNSSGSELYYSTVSESP-----									
C. plantaginaceum 2	SAG-----	LSSEVRKVFVAENE-RAEGSVGFILSTMTLSEVDSLLDGGGLRPADFAFTCNSSGSELYYSTVSESP-----									
Vicia faba	LKGNPKMILSIQIIVRAVRLDPQMSRFGSALSTAMPVLAELADFLKAGDVKNDFDALICSSGSELYYSTVSESP-----										
S. tuberosom	TSG-----	LLQVKTIVISIGVEQ-RPTGSGIFILSTMTLSEVDSLLDGGGLRPADFAFTCNSSGSELYYSTVSESP-----									
Beta vulgaris	SSG-----	LSSEVRKVFVAENE-RAEGSVGFILSTMTLSEVDSLLDGGGLRPADFAFTCNSSGSELYYSTVSESP-----									
Zea mays	EDG-----	LDIVRRIIDRAGKE-KIEGSGIFILSTMTLSEVDSLLDGGGLRPADFAFTCNSSGSELYYSTVSESP-----									
Oryza sativa 1	DDGRSKMLQVIOEVRARVSDQMSRISGFSALSTAMPVLAELADFLKAGDVKNDFDALICSSGSELYYSTVSESP-----										
Oryza sativa 2	DDGRSKMLQVIOEVRARVSDQMSRISGFSALSTAMPVLAELADFLKAGDVKNDFDALICSSGSELYYSTVSESP-----										
A. thaliana 1	DDGRSKMLQVIOEVRARVSDQMSRISGFSALSTAMPVLAELADFLKAGDVKNDFDALICSSGSELYYSTVSESP-----										
A. thaliana 2	DDGRSKMLQVIOEVRARVSDQMSRISGFSALSTAMPVLAELADFLKAGDVKNDFDALICSSGSELYYSTVSESP-----										
S. officinarum	DDGRSKMLQVIOEVRARVSDQMSRISGFSALSTAMPVLAELADFLKAGDVKNDFDALICSSGSELYYSTVSESP-----										

FIGURE 3 (continued)

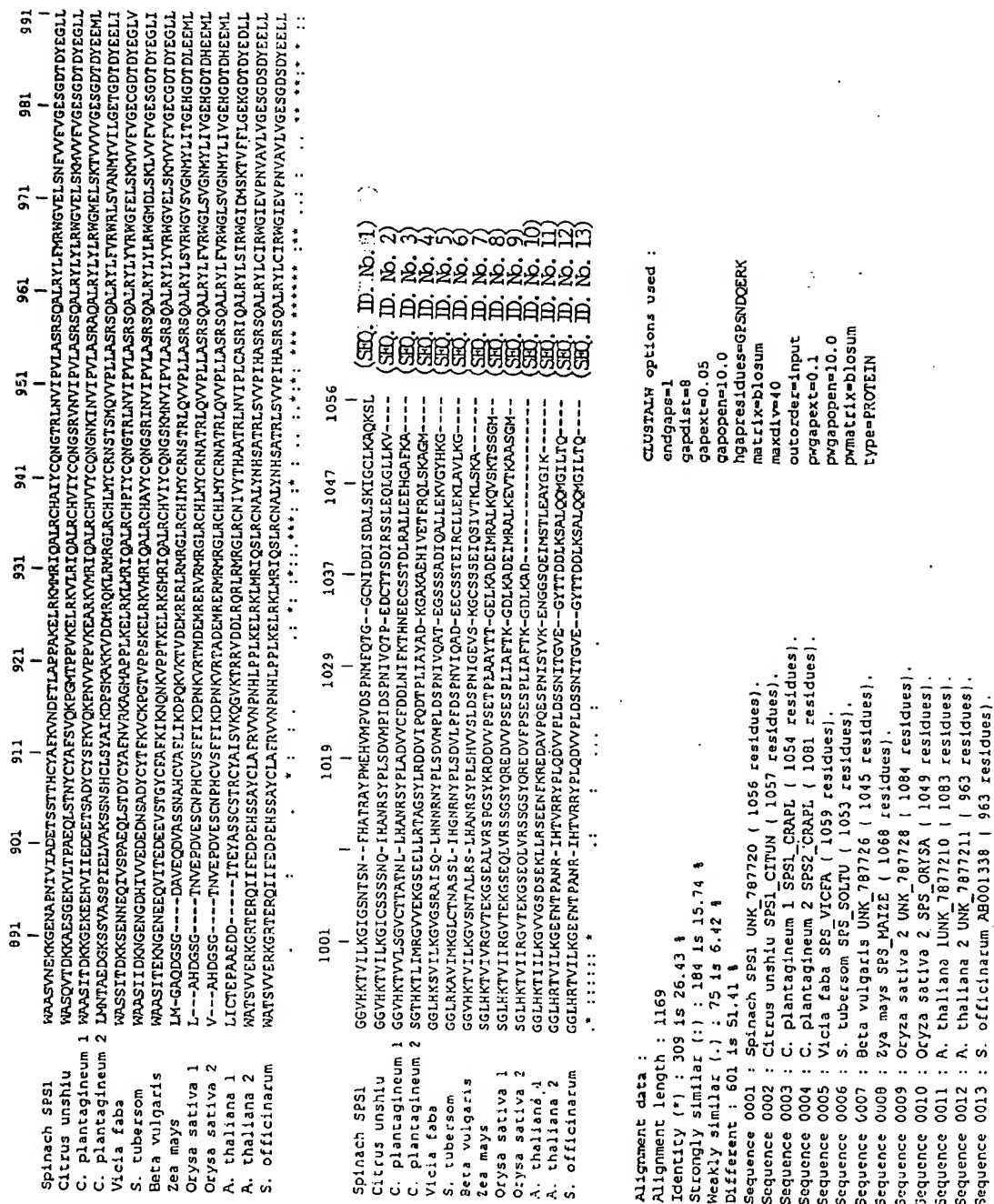


FIGURE 3 (continued)

Spinach sps1 vs Synechocystis

Spinach sps1 Synechocystis	10	20	30	40	50	60	70	80	90	100	110
	MAGNDWINSYLEAILDVGQGI	DASTGKTSTAPPSILLRERGH	FSRYSVEEISG	FDTDLHRSVRAASTRS	POERTRLNLCWR	IWNLARKKKQIEGE	EAQRLAK				
Spinach sps1 Synechocystis	120	130	140	150	160	170	180	190	200	210	220
	RHVERERGRREATADMS	EDLSEGERGTVADM	FASESTKGRMRIS	SSVMQNWANT	TEKKLYVLLISLH	GIENNELGRDS	DTGGQVKYV	VELARALGSM	PGVYR		
Spinach sps1 Synechocystis	230	240	250	260	270	280	290	300	310	320	330
	VOLLTRQVSAPGV	WMSYGEPT	MLSSRNSENTE	QLGSSGAYIIRI	PEFGPKDYAK	ELLWPYIPE	FVDGALSHIK	QSKVLG	GEIGGGL	PWNPASVHGHY	ADAGDSAA
	VOLLTRLIKDPKY	DADYAPRELIGD	-----	-----	-----	-----	-----	-----	-----	-----	-----
Spinach sps1 Synechocystis	340	350	360	370	380	390	400	410	420	430	440
	LLSGALNVP	MVFTGHS	GRDKLDQL	KQGRLSREE	VDATYKIM	RRIAEELCL	DASEIVIT	STROEIEE	QOLYHGF	DLVLERKLR	ARMRRGV
	RLSHOLG	IPLVHTG	SLGRSK	TRLLSG	-----	-----	-----	-----	-----	-----	-----
Spinach sps1 Synechocystis	450	460	470	480	490	500	510	520	530	540	550
	EFNHIAP	EDADMDT	DIDGHKES	NANPD	PVIMSEIM	RFFSNGRK	PMILALARP	DPKKNLT	TLVKA	FGECRPL	RELANLTL
	DLEKFP	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Spinach sps1 Synechocystis	560	570	580	590	600	610	620	630	640	650	659
	YGQVAYPKH	KQSDVP	DIYRLA	KTGVIN	PAFIE	FEGLT	IEAAYGL	PVATNG	GPVDI	IGVLD	NGLLID
	YGVAYPK	QNOA	EDVYAL	FRLTAL	SGVFIN	PALEPE	GLTIEA	ACGVP	IVATED	GGPVDI	IKNCQ
Spinach sps1 Synechocystis	669	679	689	699	709	719	729	739	749	759	769
	SWPEHCKN	YLSRI	ASCKP	ROP	NMORI	DEGS	NSDTS	AGDSLR	DIQDIS	LNKL	SLDA
	SWP	SHVES	YLEA	INALT	QOTS	VLRSD	-----	-----	-----	-----	-----
Spinach sps1 Synechocystis	779	789	799	809	819	829	839	849	859	869	879
	RKCI	FVIAL	CDVTS	DLLQ	VIKTV	ISIVG	QRTG	SIGFT	SMTL	SEVDS	LLD
	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Spinach sps1 Synechocystis	889	899	909	919	929	939	949	959	969	979	989
	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

FIGURE 4

FIGURE 4 (continued)

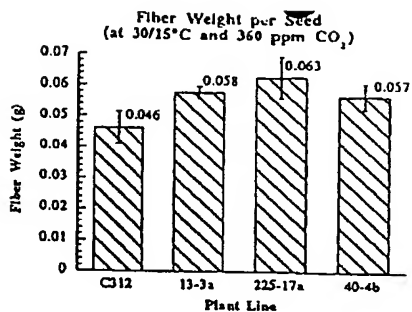


FIGURE 5

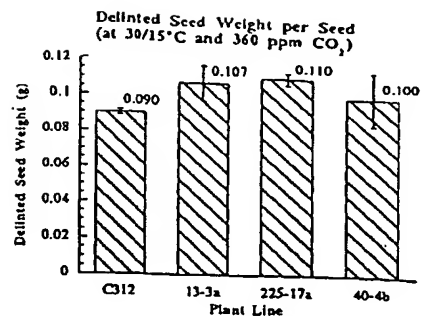


FIGURE 6

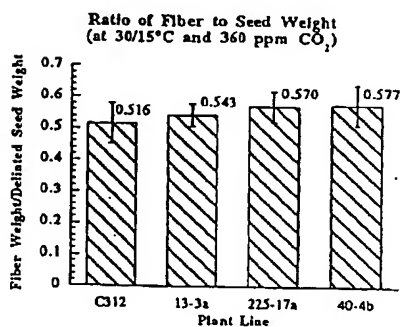


FIGURE 7

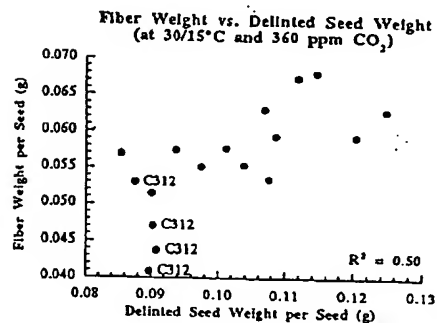


FIGURE 8

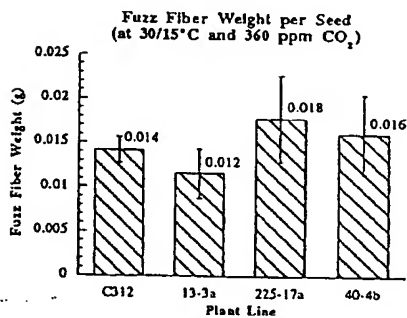


FIGURE 9

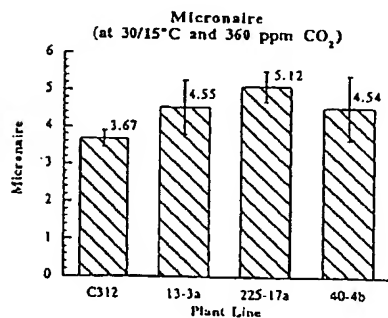


FIGURE 10

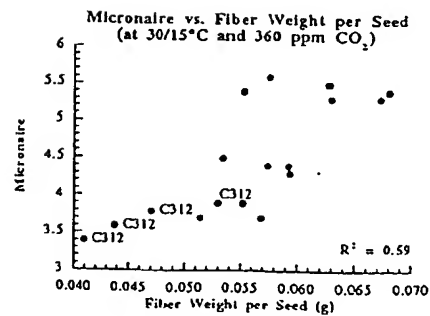


FIGURE 11

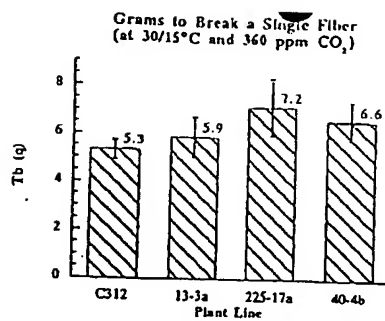


FIGURE 12

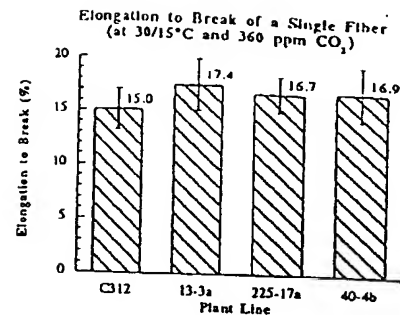


FIGURE 13

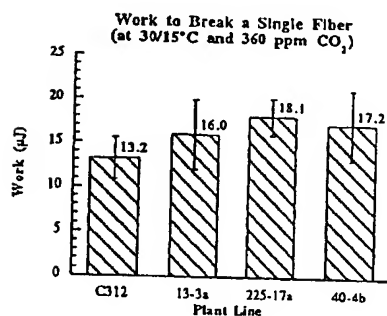


FIGURE 14

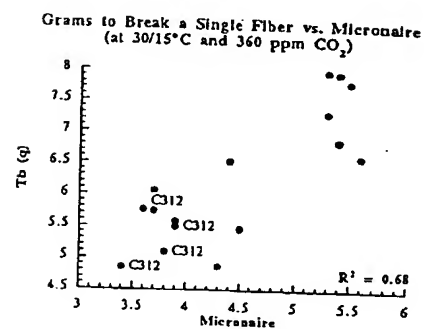


FIGURE 15

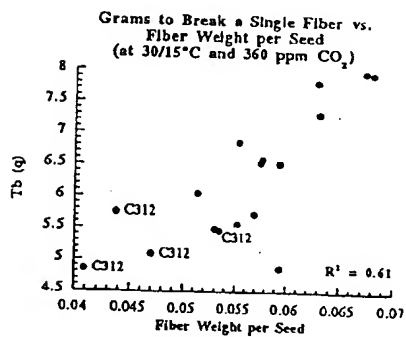


FIGURE 16

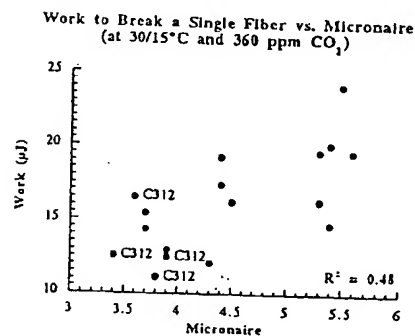


FIGURE 17

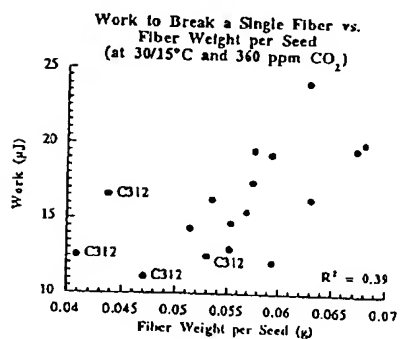


FIGURE 18

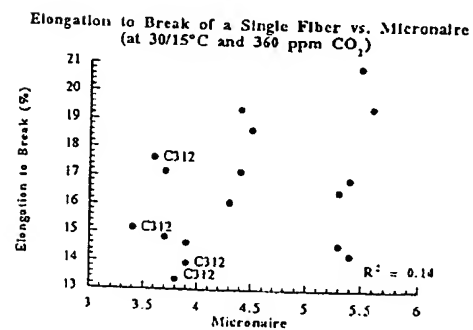


FIGURE 19

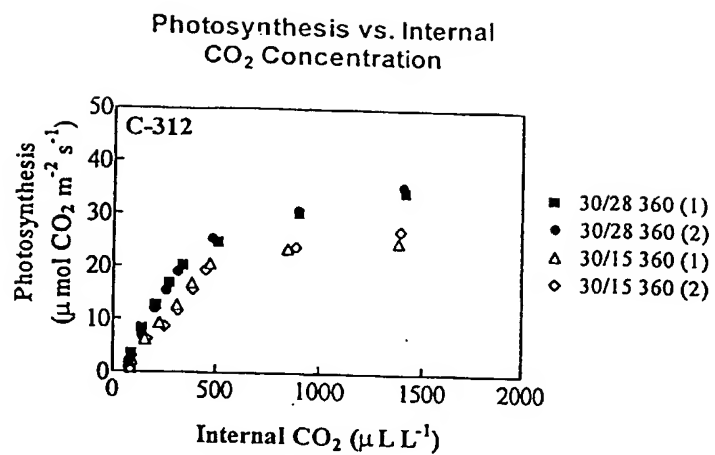


FIGURE 20

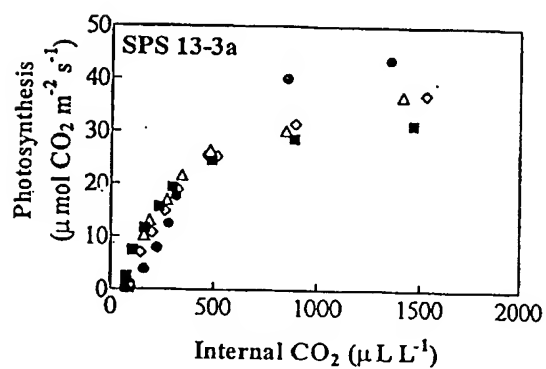


FIGURE 21

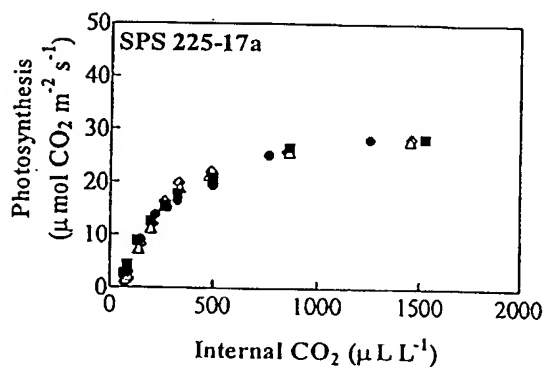


FIGURE 22



## SEQUENCE LISTING

&lt;110&gt; Texas Tech University

<120> TRANSGENIC FIBER PRODUCING PLANTS WITH INCREASED  
EXPRESSION OF SUCROSE PHOSPHATE SYNTHASE

&lt;130&gt; 201304/1001

&lt;140&gt;

&lt;141&gt;

&lt;150&gt; 09/394,272

&lt;151&gt; 1999-09-10

&lt;160&gt; 14

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 1056

&lt;212&gt; PRT

&lt;213&gt; Spinacia oleracea

&lt;400&gt; 1

Met	Ala	Gly	Asn	Asp	Trp	Ile	Asn	Ser	Tyr	Leu	Glu	Ala	Ile	Leu	Asp
1				5					10					15	

Val	Gly	Gly	Gln	Gly	Ile	Asp	Ala	Ser	Thr	Gly	Lys	Thr	Ser	Thr	Ala
			20					25					30		

Pro	Pro	Ser	Leu	Leu	Leu	Arg	Glu	Arg	Gly	His	Phe	Ser	Pro	Ser	Arg
		35					40					45			

Tyr	Phe	Val	Glu	Glu	Val	Ile	Ser	Gly	Phe	Asp	Glu	Thr	Asp	Leu	His
	50					55					60				

Arg	Ser	Trp	Val	Arg	Ala	Ala	Ser	Thr	Arg	Ser	Pro	Gln	Glu	Arg	Asn
65					70					75					80

Thr	Arg	Leu	Glu	Asn	Leu	Cys	Trp	Arg	Ile	Trp	Asn	Leu	Ala	Arg	Lys
				85					90					95	

Lys	Lys	Gln	Ile	Glu	Gly	Glu	Glu	Ala	Gln	Arg	Leu	Ala	Lys	Arg	His
			100					105					110		

Val	Glu	Arg	Glu	Arg	Gly	Arg	Arg	Glu	Ala	Thr	Ala	Asp	Met	Ser	Glu
		115					120					125			

Asp Leu Ser Glu Gly Glu Arg Gly Asp Thr Val Ala Asp Met Leu Phe  
 130 135 140

Ala Ser Glu Ser Thr Lys Gly Arg Met Arg Arg Ile Ser Ser Val Glu  
 145 150 155 160

Met Met Asp Asn Trp Ala Asn Thr Phe Lys Glu Lys Lys Leu Tyr Val  
 165 170 175

Val Leu Ile Ser Leu His Gly Leu Ile Arg Gly Glu Asn Met Glu Leu  
 180 185 190

Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys Tyr Val Val Glu Leu  
 195 200 205

Ala Arg Ala Leu Gly Ser Met Pro Gly Val Tyr Arg Val Asp Leu Leu  
 210 215 220

Thr Arg Gln Val Ser Ala Pro Gly Val Asp Trp Ser Tyr Gly Glu Pro  
 225 230 235 240

Thr Glu Met Leu Ser Ser Arg Asn Ser Glu Asn Ser Thr Glu Gln Leu  
 245 250 255

Gly Glu Ser Ser Gly Ala Tyr Ile Ile Arg Ile Pro Phe Gly Pro Lys  
 260 265 270

Asp Lys Tyr Val Ala Lys Glu Leu Leu Trp Pro Tyr Ile Pro Glu Phe  
 275 280 285

Val Asp Gly Ala Leu Ser His Ile Lys Gln Met Ser Lys Val Leu Gly  
 290 295 300

Glu Gln Ile Gly Gly Gly Leu Pro Val Trp Pro Ala Ser Val His Gly  
 305 310 315 320

His Tyr Ala Asp Ala Gly Asp Ser Ala Ala Leu Leu Ser Gly Ala Leu  
 325 330 335

Asn Val Pro Met Val Phe Thr Gly His Ser Leu Gly Arg Asp Lys Leu  
 340 345 350

Asp Gln Leu Leu Lys Gln Gly Arg Leu Ser Arg Glu Glu Val Asp Ala  
 355 360 365

Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala Glu Glu Leu Cys Leu Asp  
 370 375 380

Ala Ser Glu Ile Val Ile Thr Ser Thr Arg Gln Glu Ile Glu Glu Gln  
 385 390 395 400  
 Trp Gln Leu Tyr His Gly Phe Asp Leu Val Leu Glu Arg Lys Leu Arg  
 405 410 415  
 Ala Arg Met Arg Arg Gly Val Ser Cys His Gly Arg Phe Met Pro Arg  
 420 425 430  
 Met Ala Lys Ile Pro Pro Gly Met Glu Phe Asn His Ile Ala Pro Glu  
 435 440 445  
 Asp Ala Asp Met Asp Thr Asp Ile Asp Gly His Lys Glu Ser Asn Ala  
 450 455 460  
 Asn Pro Asp Pro Val Ile Trp Ser Glu Ile Met Arg Phe Phe Ser Asn  
 465 470 475 480  
 Gly Arg Lys Pro Met Ile Leu Ala Leu Ala Arg Pro Asp Pro Lys Lys  
 485 490 495  
 Asn Leu Thr Thr Leu Val Lys Ala Phe Gly Glu Cys Arg Pro Leu Arg  
 500 505 510  
 Glu Leu Ala Asn Leu Thr Leu Ile Ile Gly Asn Arg Asp Asp Ile Asp  
 515 520 525  
 Glu Met Ser Thr Thr Ser Ser Ser Val Leu Ile Ser Ile Leu Lys Leu  
 530 535 540  
 Ile Asp Lys Tyr Asp Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His  
 545 550 555 560  
 Lys Gln Ser Asp Val Pro Asp Ile Tyr Arg Leu Ala Ala Lys Thr Lys  
 565 570 575  
 Gly Val Phe Ile Asn Pro Ala Phe Ile Glu Pro Phe Gly Leu Thr Leu  
 580 585 590  
 Ile Glu Ala Ala Ala Tyr Gly Leu Pro Ile Val Ala Thr Lys Asn Gly  
 595 600 605  
 Gly Pro Val Asp Ile Ile Gly Val Leu Asp Asn Gly Leu Leu Ile Asp  
 610 615 620  
 Pro His Asp Gln Lys Ser Ile Ala Asp Ala Leu Leu Lys Leu Val Ala  
 625 630 635 640

Asp Lys His Leu Trp Thr Lys Cys Arg Gln Asn Gly Leu Lys Asn Ile  
 645 650 655  
 His Leu Phe Ser Trp Pro Glu His Cys Lys Asn Tyr Leu Ser Arg Ile  
 660 665 670  
 Ala Ser Cys Lys Pro Arg Gln Pro Asn Trp Gln Arg Ile Asp Glu Gly  
 675 680 685  
 Ser Glu Asn Ser Asp Thr Asp Ser Ala Gly Asp Ser Leu Arg Asp Ile  
 690 695 700  
 Gln Asp Ile Ser Leu Asn Leu Lys Leu Ser Leu Asp Ala Glu Arg Thr  
 705 710 715 720  
 Glu Gly Gly Asn Ser Phe Asp Asp Ser Leu Asp Ser Glu Glu Ala Asn  
 725 730 735  
 Ala Lys Arg Lys Ile Glu Asn Ala Val Ala Lys Leu Ser Lys Ser Met  
 740 745 750  
 Asp Lys Ala Gln Val Asp Val Gly Asn Leu Lys Phe Pro Ala Ile Arg  
 755 760 765  
 Arg Arg Lys Cys Ile Phe Val Ile Ala Leu Asp Cys Asp Val Thr Ser  
 770 775 780  
 Asp Leu Leu Gln Val Ile Lys Thr Val Ile Ser Ile Val Gly Glu Gln  
 785 790 795 800  
 Arg Pro Thr Gly Ser Ile Gly Phe Ile Leu Ser Thr Ser Met Thr Leu  
 805 810 815  
 Ser Glu Val Asp Ser Leu Leu Asp Ser Gly Gly Leu Arg Pro Ala Asp  
 820 825 830  
 Phe Asp Ala Phe Ile Cys Asn Ser Gly Ser Glu Leu Tyr Tyr Pro Ser  
 835 840 845  
 Thr Asp Tyr Ser Glu Ser Pro Phe Val Leu Asp Gln Asp Tyr Tyr Ser  
 850 855 860  
 His Ile Asp Tyr Arg Trp Gly Gly Glu Gly Leu Trp Lys Thr Leu Val  
 865 870 875 880  
 Lys Trp Ala Ala Ser Val Asn Glu Lys Lys Gly Glu Asn Ala Pro Asn  
 885 890 895

Ile Val Ile Ala Asp Glu Thr Ser Ser Thr Thr His Cys Tyr Ala Phe  
                   900                                  905                                  910  
 Lys Val Asn Asp Phe Thr Leu Ala Pro Pro Ala Lys Glu Leu Arg Lys  
                   915                                  920                                  925  
 Met Met Arg Ile Gln Ala Leu Arg Cys His Ala Ile Tyr Cys Gln Asn  
                   930                                  935                                  940  
 Gly Thr Arg Leu Asn Val Ile Pro Val Leu Ala Ser Arg Ser Gln Ala  
 945                                  950                                  955                                  960  
 Leu Arg Tyr Leu Phe Met Arg Trp Gly Val Glu Leu Ser Asn Phe Val  
                                   965                                  970                                  975  
 Val Phe Val Gly Glu Ser Gly Asp Thr Asp Tyr Glu Gly Leu Leu Gly  
                                   980                                  985                                  990  
 Gly Val His Lys Thr Val Ile Leu Lys Gly Ile Gly Ser Asn Thr Ser  
                   995                                  1000                                  1005  
 Asn Phe His Ala Thr Arg Ala Tyr Pro Met Glu His Val Met Pro Val  
                   1010                                  1015                                  1020  
 Asp Ser Pro Asn Met Phe Gln Thr Gly Gly Cys Asn Ile Asp Asp Ile  
 1025                                  1030                                  1035                                  1040  
 Ser Asp Ala Leu Ser Lys Ile Gly Cys Leu Lys Ala Gln Lys Ser Leu  
                   1045                                  1050                                  1055

&lt;210&gt; 2

&lt;211&gt; 1057

&lt;212&gt; PRT

&lt;213&gt; Citrus unshiu

&lt;400&gt; 2

Met Ala Gly Asn Asp Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu Asp  
   1                                  5                                  10                                  15  
 Val Gly Pro Gly Leu Asp Asp Ala Lys Ser Ser Leu Leu Leu Arg Glu  
                   20                                  25                                  30  
 Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu Val Ile Thr

35	40	45
Gly Phe Asp Glu Thr Asp Leu His Arg Ser Trp Val Lys Ala Gln Ala		
50	55	60
Thr Arg Ser Pro Gln Glu Arg Asn Thr Arg Leu Glu Asn Met Cys Trp		
65	70	75 80
Arg Ile Trp Asn Leu Ala Arg Gln Lys Lys Gln Leu Glu Gly Glu Ala		
	85	90 95
Ala Gln Arg Met Ala Lys Arg Arg Leu Glu Arg Glu Arg Gly Arg Arg		
	100	105 110
Glu Ala Thr Ala Asp Met Ser Glu Asp Leu Ser Glu Gly Glu Lys Gly		
115	120	125
Asp Ile Val Ser Asp Val Ser Ala His Gly Asp Ser Thr Arg Ser Arg		
130	135	140
Leu Pro Arg Ile Ser Ser Val Asp Ala Met Glu Thr Trp Ile Ser Gln		
145	150	155 160
Gln Lys Gly Lys Lys Leu Tyr Ile Val Leu Ile Ser Ile His Gly Leu		
	165	170 175
Ile Arg Gly Glu Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly		
	180	185 190
Gln Val Lys Tyr Val Val Glu Leu Ala Arg Ala Leu Gly Ser Met Pro		
	195	200 205
Gly Val Tyr Arg Val Asp Leu Leu Thr Arg Gln Val Ser Ala Pro Asp		
210	215	220
Val Asp Trp Ser Tyr Gly Glu Pro Thr Glu Met Leu Thr Pro Arg Asn		
225	230	235 240
Ser Asp Asp Phe Met Asp Asp Met Gly Glu Ser Ser Gly Ala Tyr Ile		
	245	250 255
Ile Arg Ile Pro Phe Gly Pro Lys Asp Lys Tyr Ile Ala Lys Glu Leu		
	260	265 270
Leu Trp Pro His Ile Pro Glu Phe Val Asp Gly Ala Leu Asn His Ile		
	275	280 285
Ile Arg Met Ser Asn Val Leu Gly Glu Gln Ile Gly Gly Gly Lys Pro		

290	295	300
Val Trp Pro Val Ala Ile His Gly His Tyr Ala Asp Ala Gly Asp Ser		
305	310	315 320
Ala Ala Leu Leu Ser Gly Ala Leu Asn Val Pro Met Leu Phe Thr Gly		
	325	330 335
His Ser Leu Gly Arg Asp Lys Leu Glu Gln Leu Leu Lys Gln Ala Arg		
	340	345 350
Leu Ser Arg Asp Glu Ile Asn Ala Thr Tyr Lys Ile Met Arg Arg Ile		
	355	360 365
Glu Ala Glu Glu Leu Ser Leu Asp Ala Ser Glu Ile Val Ile Thr Ser		
	370	375 380
Thr Arg Gln Glu Ile Glu Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp		
	385	390 395 400
Pro Val Leu Glu Arg Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser		
	405	410 415
Cys Tyr Gly Lys Phe Met Pro Arg Met Ala Ile Ile Pro Pro Gly Met		
	420	425 430
Glu Phe His His Ile Val Pro Gln Asp Gly Asp Met Asp Gly Glu Thr		
	435	440 445
Glu Gly Asn Glu Asp Asn Pro Ala Ser Pro Asp Pro Pro Ile Trp Ser		
	450	455 460
Glu Ile Met Arg Phe Phe Thr Asn Pro Arg Lys Pro Val Ile Leu Ala		
	465	470 475 480
Leu Ala Arg Pro Asp Pro Lys Lys Asn Ile Thr Thr Leu Val Lys Ala		
	485	490 495
Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu Thr Leu Ile		
	500	505 510
Met Gly Asn Arg Asp Gly Ile Asp Glu Met Ser Ser Thr Ser Ala Ser		
	515	520 525
Val Leu Leu Ser Val Leu Lys Leu Ile Asp Lys Tyr Asp Leu Tyr Gly		
	530	535 540
Gln Val Ala Tyr Pro Lys His His Lys Gln Ser Asp Val Pro Glu Ile		

545	550	555	560
Tyr Arg Leu Ala Ala Lys Thr Lys Gly Val Phe Ile Asn Pro Ala Phe	565	570	575
Ile Glu Pro Phe Gly Leu Thr Leu Ile Glu Ala Ala Ala His Gly Leu	580	585	590
Pro Ile Val Ala Thr Lys Asn Gly Gly Pro Val Asp Ile His Arg Val	595	600	605
Leu Asp Asn Gly Leu Leu Val Asp Pro His Asp Gln Gln Ser Ile Ala	610	615	620
Asp Ala Leu Leu Lys Leu Val Ala Gly Lys Gln Leu Trp Ala Arg Cys	625	630	635
Arg Gln Asn Gly Leu Lys Asn Ile His Leu Phe Ser Trp Pro Glu His	645	650	655
Cys Lys Thr Tyr Leu Ser Arg Ile Ala Gly Cys Lys Pro Arg His Pro	660	665	670
Gln Trp Gln Arg Thr Asp Asp Gly Gly Glu Thr Ser Glu Ser Asp Ser	675	680	685
Pro Gly Asp Ser Leu Arg Asp Ile Gln Asp Ile Ser Leu Asn Leu Lys	690	695	700
Phe Ser Leu Asp Gly Glu Lys Ser Gly Ala Ser Gly Asn Asp Asp Ser	705	710	715
Leu Asp Ser Glu Gly Asn Val Ala Asp Arg Lys Ser Arg Leu Glu Asn	725	730	735
Ala Val Leu Ala Trp Ser Lys Gly Val Leu Lys Asp Thr Arg Lys Ser	740	745	750
Gly Ser Thr Asp Lys Val Asp Gln Asn Thr Gly Ala Ala Lys Phe Pro	755	760	765
Ala Leu Arg Arg Arg Lys His Ile Phe Val Ile Ser Val Asp Cys Asp	770	775	780
Ser Thr Thr Gly Leu Leu Asp Ala Thr Lys Lys Ile Cys Glu Ala Val	785	790	795
Glu Lys Glu Arg Thr Glu Gly Ser Ile Gly Phe Ile Leu Ser Thr Ser			800



805	810	815
Met Thr Ile Ser Glu Ile His Ser Phe Leu Val Ser Gly His Leu Ser 820	825	830
Pro Ser Asp Phe Asp Ala Phe Ile Cys Asn Ser Gly Ser Asp Leu Tyr 835	840	845
Tyr Ser Thr Leu Asn Ser Glu Asp Gly Pro Phe Val Val Asp Phe Tyr 850	855	860
Tyr His Ser His Ile Glu Tyr Arg Trp Gly Gly Glu Gly Leu Arg Lys 865	870	875 880
Thr Leu Val Arg Trp Ala Ser Gln Val Thr Asp Lys Lys Ala Glu Ser 885	890	895
Gly Glu Lys Val Leu Thr Pro Ala Glu Gln Leu Ser Thr Asn Tyr Cys 900	905	910
Tyr Ala Phe Ser Val Gln Lys Pro Gly Met Thr Pro Pro Val Lys Glu 915	920	925
Leu Arg Lys Val Leu Arg Ile Gln Ala Leu Arg Cys His Val Ile Tyr 930	935	940
Cys Gln Asn Gly Ser Arg Val Asn Val Ile Pro Val Leu Ala Ser Arg 945	950	955 960
Ser Gln Ala Leu Arg Tyr Leu Tyr Leu Arg Trp Gly Val Glu Leu Ser 965	970	975
Lys Met Val Val Phe Val Gly Glu Ser Gly Asp Thr Asp Tyr Glu Gly 980	985	990
Leu Leu Gly Gly Val His Lys Thr Val Ile Leu Lys Gly Ile Cys Ser 995	1000	1005
Ser Ser Ser Asn Gln Ile His Ala Asn Arg Ser Tyr Pro Leu Ser Asp 1010	1015	1020
Val Met Pro Ile Asp Ser Pro Asn Ile Val Gln Thr Pro Glu Asp Cys 1025	1030	1035 1040
Thr Thr Ser Asp Ile Arg Ser Ser Leu Glu Gln Leu Gly Leu Leu Lys 1045	1050	1055
Val		

&lt;210&gt; 3

&lt;211&gt; 1054

&lt;212&gt; PRT

<213> *Craterostigma plantagineum*

&lt;400&gt; 3

Met Ala Gly Asn Asp Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu Asp  
 1 5 10 15

Val Gly Pro Gly Ile Asp Glu Ala Lys Gly Ser Leu Leu Leu Arg Glu  
 20 25 30

Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu Val Val Ser  
 35 40 45

Gly Phe Asp Glu Thr Asp Leu His Arg Ser Trp Ile Arg Ala Gln Ala  
 50 55 60

Thr Arg Ser Pro Gln Glu Arg Asn Thr Arg Leu Glu Asn Met Cys Trp  
 65 70 75 80

Arg Ile Trp Asn Leu Ala Arg Gln Lys Lys Gln Leu Glu Asn Glu Glu  
 85 90 95

Ala Gln Arg Met Ala Lys Arg Arg Leu Glu Arg Glu Arg Gly Arg Arg  
 100 105 110

Glu Ala Val Ala Asp Met Ser Glu Asp Leu Ser Glu Gly Glu Lys Gly  
 115 120 125

Asp Ile Val Val Asp His Ser His His Gly Glu Ser Asn Arg Gly Arg  
 130 135 140

Leu Pro Arg Ile Asn Ser Val Asp Thr Met Glu Ala Trp Met Asn Gln  
 145 150 155 160

Gln Lys Gly Lys Lys Leu Tyr Ile Val Leu Ile Ser Leu His Gly Leu  
 165 170 175

Ile Arg Gly Glu Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly  
 180 185 190

Gln Val Lys Tyr Val Val Glu Leu Ala Arg Ala Leu Gly Ser Met Pro  
 195 200 205

Gly Val Tyr Arg Val Asp Leu Leu Thr Arg Gln Val Ser Ser Pro Glu  
 210 215 220  
 Val Asp Trp Ser Tyr Gly Glu Pro Thr Glu Met Leu Pro Pro Arg Asn  
 225 230 235 240  
 Ser Glu Asn Met Met Asp Glu Met Gly Glu Ser Ser Gly Ser Tyr Ile  
 245 250 255  
 Val Arg Ile Pro Phe Gly Pro Lys Asp Lys Tyr Val Ala Lys Glu Leu  
 260 265 270  
 Leu Trp Pro His Ile Pro Glu Phe Val Asp Gly Ala Leu Gly His Ile  
 275 280 285  
 Ile Gln Met Ser Lys Val Leu Gly Glu Gln Ile Gly Asn Gly His Pro  
 290 295 300  
 Ile Trp Pro Ala Ala Ile His Gly His Tyr Ala Asp Ala Gly Asp Ser  
 305 310 315 320  
 Ala Ala Leu Leu Ser Gly Ala Leu Asn Val Pro Met Leu Phe Thr Gly  
 325 330 335  
 His Ser Leu Gly Arg Asp Lys Leu Glu Gln Leu Leu Arg Gln Gly Arg  
 340 345 350  
 Leu Ser Arg Asp Glu Ile Asn Ser Thr Tyr Lys Ile Met Arg Arg Ile  
 355 360 365  
 Glu Ala Glu Glu Leu Ser Leu Asp Ala Ser Glu Met Val Ile Thr Ser  
 370 375 380  
 Thr Arg Gln Glu Ile Glu Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp  
 385 390 395 400  
 Pro Ile Leu Glu Arg Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser  
 405 410 415  
 Cys Tyr Gly Arg Phe Met Pro Arg Met Met Val Ile Pro Pro Gly Met  
 420 425 430  
 Glu Phe His His Ile Val Pro His Asp Gly Asp Leu Asp Ala Glu Pro  
 435 440 445  
 Glu Phe Asn Glu Asp Ser Lys Ser Pro Asp Pro His Ile Trp Thr Glu  
 450 455 460

Ile Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Met Ile Leu Ala Leu  
 465 470 475 480

Ala Arg Pro Asp Pro Lys Lys Asn Leu Thr Thr Leu Val Lys Ala Phe  
 485 490 495

Gly Glu Cys Lys Pro Leu Arg Glu Leu Ala Asn Leu Thr Leu Ile Met  
 500 505 510

Gly Asn Arg Asp Asn Ile Asp Glu Met Ser Gly Thr Asn Ala Ser Val  
 515 520 525

Leu Leu Ser Ile Leu Lys Met Ile Asp Lys Tyr Asp Leu Tyr Gly Leu  
 530 535 540

Val Ala Tyr Pro Lys His His Lys Gln Ser Asp Val Pro Asp Ile Tyr  
 545 550 555 560

Arg Leu Ala Ala Lys Thr Lys Gly Val Phe Ile Asn Pro Ala Phe Ile  
 565 570 575

Glu Pro Phe Gly Leu Thr Leu Ile Glu Ala Ala Ala His Gly Leu Pro  
 580 585 590

Ile Val Ala Thr Lys Asn Gly Gly Pro Val Asp Ile His Arg Val Leu  
 595 600 605

Asp Asn Gly Ile Leu Val Asp Pro His Asn Gln Glu Ser Ile Ala Asp  
 610 615 620

Ala Leu Leu Lys Leu Val Ala Glu Lys His Leu Trp Ala Lys Cys Arg  
 625 630 635 640

Ala Asn Gly Leu Lys Asn Ile His Leu Phe Ser Trp Pro Glu His Cys  
 645 650 655

Lys Ser Tyr Leu Ser Lys Leu Ala Ser Cys Lys Pro Arg Gln Pro Arg  
 660 665 670

Trp Leu Arg Asn Glu Glu Asp Asp Asp Glu Asn Ser Glu Ser Asp Ser  
 675 680 685

Pro Ser Asp Ser Leu Arg Asp Ile Gln Asp Ile Ser Leu Asn Leu Lys  
 690 695 700

Phe Ser Phe Asp Gly Asp Lys Asn Glu Ser Arg Glu Lys Gly Gly Gly  
 705 710 715 720

Ser His Pro Asp Asp Arg Ala Ser Lys Ile Glu Asn Ala Val Leu Glu  
 725 730 735  
 Trp Ser Lys Gly Val Ala Lys Gly Pro Gln Arg Ser Met Ser Ile Glu  
 740 745 750  
 Lys Gly Glu His Asn Ser Asn Ala Gly Lys Phe Pro Ala Leu Arg Arg  
 755 760 765  
 Arg Lys Ile Met Phe Val Ile Ala Val Asp Cys Lys Pro Ser Ala Gly  
 770 775 780  
 Leu Ser Glu Ser Val Arg Lys Val Phe Ala Ala Val Glu Asn Glu Arg  
 785 790 795 800  
 Ala Glu Gly Ser Val Gly Phe Ile Leu Ala Thr Ser Phe Asn Ile Ser  
 805 810 815  
 Glu Ile Arg His Phe Leu Val Ser Glu Lys Leu Asn Pro Thr Asp Phe  
 820 825 830  
 Asp Ala Phe Ile Cys Asn Ser Gly Gly Asp Leu Tyr Tyr Ser Ser His  
 835 840 845  
 His Ser Glu Asp Asn Pro Phe Val Val Asp Leu Tyr Tyr His Ser Gln  
 850 855 860  
 Ile Glu Tyr Arg Trp Gly Gly Glu Gly Leu Arg Lys Thr Leu Val Arg  
 865 870 875 880  
 Trp Ala Ala Ser Ile Thr Asp Lys Lys Gly Glu Lys Glu Glu His Val  
 885 890 895  
 Ile Ile Glu Asp Glu Glu Thr Ser Ala Asp Tyr Cys Tyr Ser Phe Lys  
 900 905 910  
 Val Gln Lys Pro Asn Val Val Pro Pro Val Lys Glu Ala Arg Lys Val  
 915 920 925  
 Met Arg Ile Gln Ala Leu Arg Cys His Val Val Tyr Cys Gln Asn Gly  
 930 935 940  
 Asn Lys Ile Asn Val Ile Pro Val Leu Ala Ser Arg Ala Gln Ala Leu  
 945 950 955 960  
 Arg Tyr Leu Tyr Leu Arg Trp Gly Met Glu Leu Ser Lys Thr Val Val  
 965 970 975

Val Val Gly Glu Ser Gly Asp Thr Asp Tyr Glu Glu Met Leu Gly Gly  
 980 985 990  
 Val His Lys Thr Val Val Leu Ser Gly Val Cys Thr Thr Ala Thr Asn  
 995 1000 1005  
 Leu Leu His Ala Asn Arg Ser Tyr Pro Leu Ala Asp Val Val Cys Phe  
 1010 1015 1020  
 Asp Asp Leu Asn Ile Phe Lys Thr His Asn Glu Glu Cys Ser Ser Thr  
 1025 1030 1035 1040  
 Asp Leu Arg Ala Leu Leu Glu Glu His Gly Ala Phe Lys Ala  
 1045 1050  
  
 <210> 4  
 <211> 1081  
 <212> PRT  
 <213> Craterostigma plantagineum  
  
 <400> 4  
 Met Ala Gly Asn Glu Trp Ile Asn Gly Tyr Leu Glu Ala Ile Leu Asp  
 1 5 10 15  
 Thr Gly Ala Ser Ala Ile Asp Glu Asn Ser Gly Gly Gly Lys Thr Ala  
 20 25 30  
 Ala Ala Gln Lys Gly Arg His His Asp His His Phe Asn Pro Thr Lys  
 35 40 45  
 Tyr Phe Val Glu Glu Val Val Ser Gly Val Asp Glu Ser Asp Leu His  
 50 55 60  
 Arg Thr Trp Ile Lys Val Val Ala Thr Arg Asn Thr Arg Glu Arg Ser  
 65 70 75 80  
 Ser Arg Leu Glu Asn Met Cys Trp Arg Ile Trp His Leu Thr Arg Lys  
 85 90 95  
 Lys Lys Gln Leu Glu Trp Glu Asp Leu Gln Arg Leu Ala Ala Arg Lys  
 100 105 110  
 Trp Glu Arg Glu Gln Gly Arg Lys Asp Val Thr Glu Asp Met Ser Glu  
 115 120 125  
 Asp Leu Ser Glu Gly Glu Lys Gly Asp Val Met Gly Glu Thr Pro Val  
 130 135 140

Ala Leu Asp Ser Pro Arg Gly Asn Lys Lys Tyr His Arg Asn Phe Ser  
 145 150 155 160  
 Asn Leu Glu Val Trp Ser Asp Ser Asn Lys Glu Lys Lys Leu Tyr Ile  
 165 170 175  
 Val Leu Ile Ser Leu His Gly Leu Val Arg Gly Glu Asn Met Glu Leu  
 180 185 190  
 Gly Arg Asp Ser Asp Thr Gly Gly Gln Ile Lys Tyr Val Val Glu Val  
 195 200 205  
 Ala Arg Ala Leu Ala Lys Met Pro Gly Val Tyr Arg Val Asp Leu Phe  
 210 215 220  
 Thr Arg Gln Ile Ser Ser Pro Glu Val Asp Trp Ser Tyr Ala Glu Pro  
 225 230 235 240  
 Thr Glu Met Leu Ser Ser Ser Ser Thr Thr Ala Gly Glu Ala His Glu  
 245 250 255  
 Pro Glu Glu Glu Glu Glu Glu Glu Asp Leu Gly Glu Gly Ser Gly Ala  
 260 265 270  
 Tyr Ile Ile Arg Ile Pro Phe Gly Pro Arg Asp Lys Tyr Leu Arg Lys  
 275 280 285  
 Glu Leu Leu Trp Pro His Ile Gln Glu Phe Val Asp Gly Ala Leu Ser  
 290 295 300  
 His Ile Val Asn Met Ser Lys Ala Leu Gly Asp Gln Ile Gly Gly Gly  
 305 310 315 320  
 Gln Pro Val Trp Pro Tyr Val Ile His Gly His Tyr Ala Asp Ala Gly  
 325 330 335  
 Asp Ser Ala Ala Leu Leu Ser Gly Ala Leu Asn Val Pro Met Val Leu  
 340 345 350  
 Thr Gly His Ser Leu Gly Arg Asn Lys Leu Glu Gln Leu Leu Lys Gln  
 355 360 365  
 Gly Arg Gln Thr Lys Glu Asp Ile Asn Ser Met Tyr Arg Ile Met Arg  
 370 375 380  
 Arg Ile Glu Ala Glu Glu Leu Ser Leu Asp Ala Ala Glu Leu Val Ile  
 385 390 395 400

Thr Ser Thr Lys Gln Glu Ile Glu Glu Gln Trp Gly Leu Tyr Asp Gly  
 405 410 415  
 Phe Asp Val Lys Leu Glu Arg Val Leu Arg Ala Arg Ala Arg Arg Gly  
 420 425 430  
 Val Asn Cys His Gly Arg Phe Met Pro Arg Met Ala Val Ile Pro Pro  
 435 440 445  
 Gly Met Asp Phe Ser Asn Val Val Val Pro Glu Asp Gly Ser Glu Gly  
 450 455 460  
 Asp Gly Asp Leu Ala Thr Leu Thr Glu Ala Thr Ser Pro Arg Ser Val  
 465 470 475 480  
 Pro Ala Ile Trp Ala Asp Val Met Arg Phe Leu Thr Asn Pro His Lys  
 485 490 495  
 Pro Met Ile Leu Ala Leu Ser Arg Pro Asp Pro Lys Lys Asn Ile Thr  
 500 505 510  
 Thr Leu Val Lys Ala Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala  
 515 520 525  
 Asn Leu Thr Leu Ile Met Gly Asn Arg Asp Asp Ile Asp Glu Met Ser  
 530 535 540  
 Gly Gly Asn Ala Ser Val Leu Thr Thr Val Leu Lys Leu Ile Asp Arg  
 545 550 555 560  
 Tyr Asp Leu Tyr Gly Gln Val Ala Phe Pro Lys His His Lys Gln Ser  
 565 570 575  
 Asp Val Pro Glu Ile Tyr Arg Leu Ala Ser Lys Thr Lys Gly Val Phe  
 580 585 590  
 Ile Asn Pro Ala Phe Ile Glu Pro Phe Gly Leu Thr Leu Ile Glu Ala  
 595 600 605  
 Ala Ala His Gly Leu Pro Met Val Ala Thr Lys Asn Gly Gly Pro Val  
 610 615 620  
 Asp Ile His Arg Ala Leu Asn Asn Gly Leu Leu Val Asp Pro His Asp  
 625 630 635 640  
 Gln Asp Ala Ile Ala Asn Ala Leu Leu Lys Leu Val Ser Glu Lys Asn  
 645 650 655



Leu Trp Asn Glu Cys Arg Lys Asn Gly Leu Lys Asn Ile His Leu Phe  
 660 665 670  
 Ser Trp Pro Glu His Cys Arg Thr Tyr Leu Thr Arg Val Ala Ala Cys  
 675 680 685  
 Arg Met Arg His Pro Gln Trp Lys Thr Asp Thr Pro Leu Asp Glu Thr  
 690 695 700  
 Ala Ile Asp Asp Ser Leu Asn Asp Ser Leu Lys Asp Val Leu Asp Met  
 705 710 715 720  
 Ser Leu Arg Leu Ser Val Asp Gly Glu Lys Met Ser Val Asn Glu Ser  
 725 730 735  
 Ser Ser Val Glu Leu Pro Gly Gly Glu Ala Ala Glu Leu Pro Asp Gln  
 740 745 750  
 Val Arg Arg Val Leu Asn Lys Ile Lys Arg Gln Asp Ser Gly Pro Ala  
 755 760 765  
 Gln Arg Glu Ala Glu Gly Lys Ala Gly Asp Val Pro Gly Lys Tyr Pro  
 770 775 780  
 Met Leu Arg Arg Arg Arg Lys Leu Phe Val Ile Ala Leu Asp Cys Tyr  
 785 790 795 800  
 Asp Leu Lys Gly Asn Pro Asp Lys Lys Met Ile Leu Ser Ile Gln Glu  
 805 810 815  
 Ile Val Arg Ala Val Arg Leu Asp Pro Gln Met Ser Arg Phe Ser Gly  
 820 825 830  
 Phe Ala Leu Ser Thr Ala Met Pro Val Ala Glu Leu Ala Asp Phe Leu  
 835 840 845  
 Lys Ala Gly Asp Val Lys Val Asn Asp Phe Asp Ala Leu Ile Cys Ser  
 850 855 860  
 Ser Gly Ser Glu Val Tyr Tyr Pro Gly Thr Tyr Gly Glu Glu Ser Gly  
 865 870 875 880  
 Lys Leu Tyr Leu Asp Pro Asp Tyr Thr Ser His Ile Glu Tyr Arg Trp  
 885 890 895  
 Gly Gly Asp Gly Leu Lys Lys Thr Ile Ser Lys Leu Met Asn Thr Ala  
 900 905 910

Glu Asp Gly Lys Ser Ser Val Ala Ser Ser Pro Ile Glu Leu Val Ala  
 915 920 925  
 Lys Ser Ser Asn Ser His Cys Leu Ser Tyr Ala Ile Lys Asp Pro Ser  
 930 935 940  
 Lys Ala Lys Lys Val Asp Asp Met Arg Gln Lys Leu Arg Met Arg Gly  
 945 950 955 960  
 Leu Arg Cys His Leu Met Tyr Cys Arg Asn Ser Thr Ser Met Gln Val  
 965 970 975  
 Val Pro Leu Leu Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Phe Val  
 980 985 990  
 Arg Trp Arg Leu Ser Val Ala Asn Met Tyr Val Ile Leu Gly Glu Thr  
 995 1000 1005  
 Gly Asp Thr Asp Tyr Glu Glu Leu Ile Ser Gly Thr His Lys Thr Leu  
 1010 1015 1020  
 Ile Met Arg Gly Val Val Glu Lys Gly Ser Glu Glu Leu Leu Arg Thr  
 1025 1030 1035 1040  
 Ala Gly Ser Tyr Leu Arg Asp Asp Val Ile Pro Gln Asp Thr Pro Leu  
 1045 1050 1055  
 Ile Ala Tyr Ala Asp Lys Gly Ala Lys Ala Glu His Ile Val Glu Thr  
 1060 1065 1070  
 Phe Arg Gln Leu Ser Lys Ala Gly Met  
 1075 1080  
 <210> 5  
 <211> 1059  
 <212> PRT  
 <213> Vicia faba  
 <400> 5  
 Met Ala Gly Asn Asp Trp Leu Asn Ser Tyr Leu Glu Ala Ile Leu Asp  
 1 5 10 15  
 Val Gly Pro Gly Leu Asp Asp Ala Lys Ser Ser Leu Leu Leu Arg Glu  
 20 25 30  
 Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu Val Ile Gly

35	40	45
Phe Asp Glu Thr Asp Leu Tyr Arg Ser Trp Val Arg Ala Ser Ser Ser		
50	55	60
Arg Ser Pro Gln Glu Arg Asn Thr Arg Leu Glu Asn Met Cys Trp Arg		
65	70	75 80
Ile Trp Asn Leu Ala Arg Gln Lys Lys Gln Leu Glu Ser Glu Ala Val		
85	90	95
Gln Arg Val Asn Lys Arg Arg Leu Glu Arg Glu Arg Gly Arg Arg Glu		
100	105	110
Ala Thr Ala Asp Met Ser Glu Asp Leu Ser Glu Gly Glu Arg Gly Asp		
115	120	125
Pro Val Ser Asp Val Ser Thr His Gly Gly Gly Asp Ser Val Lys Ser		
130	135	140
Arg Leu Pro Arg Ile Ser Ser Ala Asp Ala Met Glu Thr Trp Val Asn		
145	150	155 160
Ser Gln Lys Gly Lys Lys Leu Tyr Ile Val Leu Ile Ser Ile His Gly		
165	170	175
Leu Ile Arg Gly Glu Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly		
180	185	190
Gly Gln Val Lys Tyr Val Val Glu Leu Ala Arg Ala Leu Gly Ser Met		
195	200	205
Pro Gly Val Tyr Arg Val Asp Leu Leu Thr Arg Gln Val Ser Ser Pro		
210	215	220
Asp Val Asp Trp Ser Tyr Gly Glu Pro Thr Glu Met Leu Ala Pro Arg		
225	230	235 240
Asn Thr Asp Glu Phe Gly Asp Asp Met Gly Glu Ser Ser Gly Ala Tyr		
245	250	255
Ile Ile Arg Ile Pro Phe Gly Pro Arg Asn Lys Tyr Ile Pro Lys Glu		
260	265	270
Glu Leu Trp Pro Tyr Ile Pro Glu Phe Val Asp Gly Ala Met Gly His		
275	280	285
Ile Ile Gln Met Ser Lys Ala Leu Gly Glu Gln Ile Gly Ser Gly His		

290	295	300
Ala Val Trp Pro Val	Ala Ile His Gly His Tyr Ala Asp Ala Gly Asp	
305	310	315 320
Ser Ala Ala Leu Leu Ser Gly Ala Leu Asn Val Pro Met Ile Phe Thr		
	325	330 335
Gly His Ser Leu Gly Arg Asp Lys Leu Glu Gln Leu Leu Lys Gln Gly		
	340	345 350
Arg Leu Ser Thr Asp Glu Ile Asn Ser Thr Tyr Lys Ile Met Arg Arg		
	355	360 365
Ile Glu Ala Glu Glu Leu Ala Leu Asp Gly Thr Glu Ile Val Ile Thr		
	370	375 380
Ser Thr Arg Gln Glu Ile Glu Glu Gln Trp Arg Leu Tyr Asn Gly Phe		
385	390	395 400
Asp Pro Val Leu Glu Arg Lys Ile Arg Ala Arg Ile Arg Arg Asn Val		
	405	410 415
Ser Cys Tyr Gly Arg Tyr Met Pro Arg Met Ser Val Ile Pro Pro Gly		
	420	425 430
Met Glu Phe His His Ile Ala Pro Leu Asp Gly Asp Ile Glu Thr Glu		
	435	440 445
Pro Glu Gly Ile Leu Asp His Pro Ala Pro Gln Asp Pro Pro Ile Trp		
	450	455 460
Ser Glu Ile Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Val Ile Leu		
465	470	475 480
Ala Leu Ala Arg Pro Asp Pro Lys Lys Asn Ile Thr Thr Leu Val Lys		
	485	490 495
Ala Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu Thr Leu		
	500	505 510
Ile Met Gly Asn Arg Asp Gly Ile Asp Glu Met Ser Ser Thr Ser Ser		
	515	520 525
Ser Val Leu Leu Ser Val Leu Lys Leu Ile Asp Lys Tyr Asp Leu Tyr		
	530	535 540
Gly Gln Val Ala Tyr Pro Lys His His Lys Gln Ser Asp Val Pro Asp		

545	550	555	560
Ile Tyr Arg Leu Ala Ala Lys Thr Lys Gly Val Phe Ile Asn Pro Ala			
565		570	575
Phe Ile Glu Pro Phe Gly Leu Thr Leu Ile Glu Ala Ala Ala Tyr Gly			
580	585		590
Leu Pro Met Val Ala Thr Lys Asn Gly Gly Pro Val Asp Ile His Arg			
595	600	605	
Val Leu Asp Asn Gly Leu Leu Ile Asp Pro His Asp Glu Lys Ser Ile			
610	615	620	
Ala Asp Ala Leu Leu Lys Leu Val Ser Asn Lys Gln Leu Trp Ala Lys			
625	630	635	640
Cys Arg Gln Asn Gly Leu Lys Asn Ile His Leu Phe Ser Trp Pro Glu			
645	650		655
His Cys Lys Thr Tyr Leu Ser Lys Ile Ala Thr Cys Lys Pro Arg His			
660	665		670
Pro Gln Trp Gln Arg Ser Glu Asp Gly Gly Glu Ser Ser Glu Ser Glu			
675	680		685
Glu Ser Pro Gly Asp Ser Leu Arg Asp Ile Gln Asp Leu Ser Leu Asn			
690	695	700	
Leu Lys Phe Ser Leu Asp Gly Glu Arg Ser Gly Asp Ser Gly Asn Asp			
705	710	715	720
Asn Ser Leu Asp Pro Asp Gly Asn Ala Thr Asp Arg Thr Thr Lys Leu			
725	730		735
Glu Asn Ala Val Leu Ser Trp Ser Lys Gly Ile Ser Lys Asp Thr Arg			
740	745		750
Arg Gly Gly Ala Thr Glu Lys Ser Gly Gln Asn Ser Asn Ala Ser Lys			
755	760	765	
Phe Pro Pro Leu Arg Ser Arg Asn Arg Leu Phe Val Ile Ala Val Asp			
770	775	780	
Cys Asp Thr Thr Ser Gly Leu Leu Glu Met Ile Lys Leu Ile Phe Glu			
785	790	795	800
Ala Ala Gly Glu Glu Arg Ala Glu Gly Ser Val Gly Phe Ile Leu Ser			

	805		810		815
Thr Ser Leu Thr Ile Ser Glu Ile Gln Ser Phe Leu Ile Ser Gly Gly	820		825		830
Leu Ser Pro Asn Asp Phe Asp Ala Tyr Ile Cys Asn Ser Gly Ser Asp	835		840		845
Leu Tyr Tyr Pro Ser Leu Asn Ser Glu Asp Arg Leu Phe Val Gly Asp	850		855		860
Leu Tyr Phe His Ser His Ile Glu Tyr Arg Trp Gly Gly Glu Gly Leu	865		870		875
					880
Arg Lys Thr Leu Ile Arg Trp Ala Ser Ser Ile Thr Asp Lys Lys Ser	885		890		895
Glu Asn Asn Glu Gln Ile Val Ser Pro Ala Glu Gln Leu Ser Thr Asp	900		905		910
Tyr Cys Tyr Ala Phe Asn Val Arg Lys Ala Gly Met Ala Pro Pro Leu	915		920		925
Lys Glu Leu Arg Lys Leu Met Arg Ile Gln Ala Leu Arg Cys His Pro	930		935		940
Ile Tyr Cys Gln Asn Gly Thr Arg Leu Asn Val Ile Pro Val Leu Ala	945		950		955
					960
Ser Arg Ser Gln Ala Leu Arg Tyr Leu Tyr Val Arg Trp Gly Phe Glu	965		970		975
Leu Ser Lys Met Val Val Phe Val Gly Glu Cys Gly Asp Thr Asp Tyr	980		985		990
Glu Gly Leu Val Gly Gly Leu His Lys Ser Val Ile Leu Lys Gly Val	995		1000		1005
Gly Ser Arg Ala Ile Ser Gln Leu His Asn Asn Arg Asn Tyr Pro Leu	1010		1015		1020
Ser Asp Val Met Pro Leu Asp Ser Pro Asn Ile Val Gln Ala Thr Glu	1025		1030		1035
					1040
Gly Ser Ser Ser Ala Asp Ile Gln Ala Leu Leu Glu Lys Val Gly Tyr	1045		1050		1055
His Lys Gly					

&lt;210&gt; 6

&lt;211&gt; 1053

&lt;212&gt; PRT

&lt;213&gt; Solanum tuberosum

&lt;400&gt; 6

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Met Ala Gly Asn Asp Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu Asp
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Val Gly Pro Gly Leu Asp Asp Lys Lys Ser Ser Leu Leu Leu Arg Glu
      20             25             30

Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu Val Ile Thr
      35             40             45

Gly Phe Asp Glu Thr Asp Leu His Arg Ser Trp Ile Arg Ala Gln Ala
  50             55             60

Thr Arg Ser Pro Gln Arg Arg Asn Thr Arg Leu Glu Asn Met Cys Trp
  65             70             75             80

Arg Ile Trp Asn Leu Ala Arg Gln Lys Lys Gln Leu Glu Gly Glu Gln
      85             90             95

Ala Gln Trp Met Ala Lys Arg Arg Gln Glu Arg Glu Arg Gly Arg Arg
      100            105            110

Glu Ala Val Ala Asp Met Ser Glu Asp Leu Ser Glu Gly Glu Lys Gly
      115            120            125

Asp Ile Val Ala Asp Met Ser Ser His Gly Glu Ser Thr Arg Gly Arg
      130            135            140

Leu Pro Arg Ile Ser Ser Val Glu Thr Met Glu Ala Trp Val Ser Gln
      145            150            155            160

Gln Arg Gly Lys Lys Leu Tyr Ile Val Leu Ile Ser Leu His Gly Leu
      165            170            175

Ile Arg Gly Glu Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly
      180            185            190

Gln Val Lys Tyr Val Val Glu Leu Ala Arg Ala Leu Gly Ser Met Pro
      195            200            205

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Gly Val Tyr Arg Val Asp Leu Leu Thr Arg Gln Val Ser Ser Pro Glu  
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Val Asp Trp Ser Tyr Gly Glu Pro Thr Glu Leu Ala Pro Ile Ser Thr  
 225 230 235 240

Asp Gly Leu Met Thr Glu Met Gly Glu Ser Ser Gly Ala Tyr Ile Ile  
 245 250 255

Arg Ile Pro Phe Gly Pro Arg Glu Lys Tyr Ile Pro Lys Glu Gln Leu  
 260 265 270

Trp Pro Tyr Ile Pro Glu Phe Val Asp Gly Ala Leu Asn His Ile Ile  
 275 280 285

Gln Met Ser Lys Val Leu Gly Glu Gln Ile Gly Ser Gly Tyr Pro Val  
 290 295 300

Trp Pro Val Ala Ile His Gly His Tyr Ala Asp Ala Gly Asp Ser Ala  
 305 310 315 320

Ala Leu Leu Ser Gly Ala Leu Asn Val Pro Met Leu Phe Thr Gly His  
 325 330 335

Ser Leu Gly Arg Asp Lys Leu Glu Gln Leu Leu Ala Gln Gly Arg Lys  
 340 345 350

Ser Lys Asp Glu Ile Asn Ser Thr Tyr Lys Ile Met Arg Arg Ile Glu  
 355 360 365

Ala Glu Glu Leu Thr Leu Asp Ala Ser Glu Ile Val Ile Thr Ser Thr  
 370 375 380

Arg Gln Glu Ile Asp Glu Gln Trp Arg Leu Tyr Asp Gly Phe Asp Pro  
 385 390 395 400

Ile Leu Glu Arg Lys Leu Arg Ala Arg Ile Lys Arg Asn Val Ser Cys  
 405 410 415

Tyr Gly Arg Phe Met Pro Arg Met Ala Val Ile Pro Pro Gly Met Glu  
 420 425 430

Phe His His Ile Val Pro His Glu Gly Asp Met Asp Gly Glu Thr Glu  
 435 440 445

Gly Ser Glu Asp Gly Lys Thr Pro Asp Pro Pro Ile Trp Ala Glu Ile  
 450 455 460



Met Arg Phe Phe Ser Asn Pro Arg Lys Pro Met Ile Leu Ala Leu Ala  
 465 470 475 480  
 Arg Pro Asp Pro Lys Lys Asn Leu Thr Thr Leu Val Lys Ala Phe Gly  
 485 490 495  
 Glu Cys Arg Pro Leu Arg Asp Leu Ala Asn Leu Thr Leu Ile Met Gly  
 500 505 510  
 Asn Arg Asp Asn Ile Asp Glu Met Ser Ser Thr Asn Ser Ala Leu Leu  
 515 520 525  
 Leu Ser Ile Leu Lys Met Ile Asp Lys Tyr Asp Leu Tyr Gly Gln Val  
 530 535 540  
 Ala Tyr Pro Lys His His Lys Gln Ser Asp Val Pro Asp Ile Tyr Arg  
 545 550 555 560  
 Leu Ala Ala Lys Thr Lys Gly Val Phe Ile Asn Pro Ala Phe Ile Glu  
 565 570 575  
 Pro Phe Gly Leu Thr Leu Ile Glu Ala Ala Ala Tyr Gly Leu Pro Met  
 580 585 590  
 Val Ala Thr Lys Asn Gly Gly Pro Val Asp Ile His Arg Val Leu Asp  
 595 600 605  
 Asn Gly Leu Leu Val Asp Pro His Asp Gln Gln Ala Ile Ala Asp Ala  
 610 615 620  
 Leu Leu Lys Leu Val Ala Asp Lys Gln Leu Trp Ala Lys Cys Arg Ala  
 625 630 635 640  
 Asn Gly Leu Lys Asn Ile His Leu Phe Ser Trp Pro Glu His Cys Lys  
 645 650 655  
 Thr Tyr Leu Ser Arg Ile Ala Ser Cys Lys Pro Arg Gln Pro Arg Trp  
 660 665 670  
 Leu Arg Ser Ile Asp Asp Asp Asp Glu Asn Ser Glu Thr Asp Ser Pro  
 675 680 685  
 Ser Asp Ser Leu Arg Asp Ile His Asp Ile Ser Leu Asn Leu Arg Phe  
 690 695 700  
 Ser Leu Asp Gly Glu Lys Asn Asp Asn Lys Glu Asn Ala Asp Asn Thr  
 705 710 715 720

Leu Asp Pro Glu Val Arg Arg Ser Lys Leu Glu Asn Ala Val Leu Ser  
 725 730 735  
 Leu Ser Lys Gly Ala Leu Lys Ser Thr Ser Lys Ser Trp Ser Ser Asp  
 740 745 750  
 Lys Ala Asp Gln Asn Pro Gly Ala Gly Lys Phe Pro Ala Ile Arg Arg  
 755 760 765  
 Arg Arg His Ile Phe Val Ile Ala Val Asp Cys Asp Ala Ser Ser Gly  
 770 775 780  
 Leu Ser Gly Ser Val Lys Lys Ile Phe Glu Ala Val Glu Lys Glu Arg  
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 Ala Glu Gly Ser Ile Gly Phe Ile Leu Ala Thr Ser Phe Asn Ile Ser  
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 Glu Val Gln Ser Phe Leu Leu Ser Glu Gly Met Asn Pro Thr Asp Phe  
 820 825 830  
 Asp Ala Tyr Ile Cys Asn Ser Gly Gly Asp Leu Tyr Tyr Ser Ser Phe  
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 His Ser Glu Gln Asn Pro Phe Val Val Asp Leu Tyr Tyr His Ser His  
 850 855 860  
 Ile Glu Tyr Arg Trp Gly Gly Glu Gly Leu Arg Lys Thr Leu Val Arg  
 865 870 875 880  
 Trp Ala Ala Ser Ile Ile Asp Lys Asn Gly Glu Asn Gly Asp His Ile  
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 Val Val Glu Asp Glu Asp Asn Ser Ala Asp Tyr Cys Tyr Thr Phe Lys  
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 Val Cys Lys Pro Gly Thr Val Pro Pro Ser Lys Glu Leu Arg Lys Val  
 915 920 925  
 Met Arg Ile Gln Ala Leu Arg Cys His Ala Val Tyr Cys Gln Asn Gly  
 930 935 940  
 Ser Arg Ile Asn Val Ile Pro Val Leu Ala Ser Arg Ser Gln Ala Leu  
 945 950 955 960  
 Arg Tyr Leu Tyr Leu Arg Trp Gly Met Asp Leu Ser Lys Leu Val Val  
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Phe Val Gly Glu Ser Gly Asp Thr Asp Tyr Glu Gly Leu Ile Gly Gly  
 980 985 990  
 Leu Arg Lys Ala Val Ile Met Lys Gly Leu Cys Thr Asn Ala Ser Ser  
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 Arg Gly Arg Phe Ser Pro Thr Arg Tyr Phe Val Glu Glu Val Ile Thr  
 35 40 45  
 Gly Phe Asp Glu Thr Asp Leu His Arg Ser Trp Val Arg Ala Gln Ala  
 50 55 60  
 Thr Arg Ser Pro Gln Glu Arg Asn Thr Arg Leu Glu Asn Met Cys Trp  
 65 70 75 80  
 Arg Ile Trp Asn Leu Ala Arg Gln Lys Lys Gln Leu Glu Asn Glu Glu  
 85 90 95  
 Ala Gln Arg Lys Thr Lys Arg Arg Met Glu Leu Glu Arg Gly Arg Arg  
 100 105 110  
 Glu Ala Thr Ala Asp Met Ser Glu Asp Leu Ser Glu Gly Glu Lys Asp  
 115 120 125  
 Ile Ser Ala His Gly Asp Ser Thr Arg Pro Arg Leu Pro Arg Ile Asn  
 130 135 140

Ser Leu Asp Ala Met Glu Thr Trp Ile Ser Gln Gln Lys Glu Lys Lys  
 145 150 155 160  
 Leu Tyr Leu Val Leu Ile Ser Leu His Gly Leu Ile Arg Gly Glu Asn  
 165 170 175  
 Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys Tyr Val  
 180 185 190  
 Val Glu Leu Ala Arg Ala Leu Gly Ser Met Pro Gly Val Tyr Arg Val  
 195 200 205  
 Asp Leu Leu Thr Arg Gln Val Ser Ser Pro Asp Val Asp Trp Ser Tyr  
 210 215 220  
 Gly Glu Pro Thr Glu Met Leu Asn Pro Arg Asp Ser Asn Gly Phe Asp  
 225 230 235 240  
 Asp Asp Asp Asp Glu Met Gly Glu Ser Ser Gly Ala Tyr Ile Val Arg  
 245 250 255  
 Ile Pro Phe Gly Pro Arg Asp Lys Tyr Ile Ala Lys Glu Glu Leu Trp  
 260 265 270  
 Pro Tyr Ile Pro Glu Phe Val Asp Gly Ala Leu Asn His Ile Val Gln  
 275 280 285  
 Met Ser Lys Val Leu Gly Glu Gln Ile Gly Ser Gly Glu Thr Val Trp  
 290 295 300  
 Pro Val Ala Ile His Gly His Tyr Ala Asp Ala Gly Asp Ser Ala Ala  
 305 310 315 320  
 Leu Leu Ser Gly Gly Leu Asn Val Pro Met Leu Leu Thr Gly His Ser  
 325 330 335  
 Leu Gly Arg Asp Lys Leu Glu Gln Leu Leu Lys Gln Gly Arg Met Ser  
 340 345 350  
 Lys Asp Asp Ile Asn Asn Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala  
 355 360 365  
 Glu Glu Leu Ser Leu Asp Ala Ser Glu Ile Val Ile Thr Ser Thr Arg  
 370 375 380  
 Gln Glu Ile Glu Glu Gln Trp His Leu Tyr Asp Gly Phe Asp Pro Val  
 385 390 395 400

Leu Glu Arg Lys Leu Arg Ala Arg Met Lys Arg Gly Val Ser Cys Tyr  
 405 410 415  
 Gly Arg Phe Met Pro Arg Met Val Val Ile Pro Pro Gly Met Glu Phe  
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 Asn His Ile Val Pro His Glu Gly Asp Met Asp Gly Glu Thr Glu Glu  
 435 440 445  
 Thr Glu Glu His Pro Thr Ser Pro Asp Pro Pro Ile Trp Ala Glu Ile  
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 Met Arg Phe Phe Ser Lys Pro Arg Lys Pro Met Ile Leu Ala Leu Ala  
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 Arg Pro Asp Pro Lys Lys Asn Ile Thr Thr Leu Val Lys Ala Phe Gly  
 485 490 495  
 Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu Thr Leu Ile Met Gly  
 500 505 510  
 Asn Arg Asp Gly Ile Asp Glu Met Ser Ser Thr Ser Ser Ser Val Leu  
 515 520 525  
 Leu Ser Val Leu Lys Leu Ile Asp Gln Tyr Asp Leu Tyr Gly Gln Val  
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 Ala Tyr Pro Lys His His Lys Gln Ala Asp Val Pro Glu Ile Tyr Arg  
 545 550 555 560  
 Leu Ala Ala Lys Thr Lys Gly Val Phe Ile Asn Pro Ala Phe Ile Glu  
 565 570 575  
 Pro Phe Gly Leu Thr Leu Ile Glu Ala Ala Ala His Gly Leu Pro Met  
 580 585 590  
 Val Ala Thr Lys Asn Gly Gly Pro Val Asp Ile Gln Arg Val Leu Asp  
 595 600 605  
 Asn Gly Leu Leu Val Asp Pro His Glu Gln Gln Ser Ile Ala Thr Ala  
 610 615 620  
 Leu Leu Lys Leu Val Ala Asp Lys Gln Leu Trp Thr Lys Cys Gln Gln  
 625 630 635 640  
 Asn Gly Leu Lys Asn Ile His Leu Tyr Ser Trp Pro Glu His Ser Lys  
 645 650 655

Thr Tyr Leu Ser Arg Ile Ala Ser Ser Arg Gln Arg Gln Pro Gln Trp  
 660 665 670  
 Gln Arg Ser Ser Asp Glu Gly Leu Asp Asn Gln Glu Pro Glu Ser Pro  
 675 680 685  
 Ser Asp Ser Leu Arg Asp Ile Lys Asp Ile Ser Leu Asn Leu Glu Val  
 690 695 700  
 Leu Val Arg Pro Glu Lys Arg Val Lys Thr Leu Lys Ile Leu Gly Leu  
 705 710 715 720  
 Met Thr Lys Ala Asn Ser Arg Met Leu Leu Cys Ser Trp Ser Asn Gly  
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 Val His Lys Met Leu Arg Lys Ala Arg Phe Ser Asp Lys Val Asp Gln  
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 Ala Ser Ser Lys Tyr Pro Ala Phe Arg Arg Arg Lys Leu Ile Tyr Val  
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 Arg Ile Phe Asp Ala Ala Gly Lys Glu Lys Ile Glu Gly Ser Ile Gly  
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 Phe Ile Leu Ser Thr Ser Tyr Ser Met Pro Glu Ile Gln Asn Tyr Leu  
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 Leu Ser Lys Gly Phe Asn Leu His Asp Phe Asp Ala Tyr Ile Cys Asn  
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 Ser Gly Ser Glu Leu Tyr Tyr Ser Ser Leu Asn Ser Glu Glu Ser Asn  
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 Gly Glu Gly Leu Arg Arg Thr Leu Leu Arg Trp Ala Ala Ser Ile Thr  
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 Glu Lys Asn Gly Glu Asn Glu Glu Gln Val Ile Thr Glu Asp Glu Glu  
 885 890 895  
 Val Ser Thr Gly Tyr Cys Phe Ala Phe Lys Ile Lys Asn Gln Asn Lys  
 900 905 910

Val Pro Pro Thr Lys Glu Leu Arg Lys Ser Met Arg Ile Gln Ala Leu  
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 Pro Val Leu Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Tyr Val Arg  
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 Trp Gly Val Glu Leu Ser Lys Met Val Val Phe Val Gly Glu Cys Gly  
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 Asp Thr Asp Tyr Glu Gly Leu Leu Gly Gly Val His Lys Thr Val Ile  
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 Ser Tyr Pro Leu Ser His Val Val Ser Leu Asp Ser Pro Asn Ile Gly  
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 Lys Leu Ser Lys Ala  
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 35 40 45  
 Phe Asn Pro Ser His Tyr Phe Val Glu Glu Val Val Lys Gly Val Asp  
 50 55 60  
 Glu Ser Asp Leu His Arg Thr Trp Ile Lys Val Val Ala Thr Arg Asn

65		70		75		80
Ala Arg Glu Arg Ser Thr Arg Leu Glu Asn Met Cys Trp Arg Ile Trp						
	85		90		95	
His Leu Ala Arg Lys Lys Lys Gln Leu Glu Leu Glu Gly Ile Gln Arg						
	100		105		110	
Ile Ser Ala Arg Arg Lys Glu Gln Glu Gln Val Arg Arg Glu Ala Thr						
	115		120		125	
Glu Asp Leu Ala Glu Asp Leu Ser Glu Gly Glu Lys Gly Asp Thr Ile						
	130		135		140	
Gly Glu Leu Ala Pro Val Glu Thr Thr Lys Lys Lys Phe Gln Arg Asn						
	145		150		155	
Phe Ser Asp Leu Thr Val Trp Ser Asp Asp Asn Lys Glu Lys Lys Leu						
	165		170		175	
Tyr Ile Val Leu Ile Ser Val His Gly Leu Val Arg Gly Glu Asn Met						
	180		185		190	
Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys Tyr Val Val						
	195		200		205	
Glu Leu Ala Arg Ala Met Ser Met Met Pro Gly Val Tyr Arg Val Asp						
	210		215		220	
Leu Phe Thr Arg Gln Val Ser Ser Pro Asp Val Asp Trp Ser Tyr Gly						
	225		230		235	
Glu Pro Thr Glu Met Leu Cys Ala Gly Ser Asn Asp Gly Glu Gly Met						
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Gly Glu Ser Gly Gly Ala Tyr Ile Val Arg Ile Pro Cys Gly Pro Arg						
	260		265		270	
Asp Lys Tyr Leu Lys Lys Glu Ala Leu Trp Pro Tyr Leu Gln Glu Phe						
	275		280		285	
Val Asp Gly Ala Leu Ala His Ile Leu Asn Met Ser Lys Ala Leu Gly						
	290		295		300	
Glu Gln Val Gly Asn Gly Arg Pro Val Leu Pro Tyr Val Ile His Gly						
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His Tyr Ala Asp Ala Gly Asp Val Ala Ala Leu Leu Ser Gly Ala Leu						



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Glu Gln Leu Leu Lys Gln Gly Arg Met Ser Lys Glu Glu Ile Asp Ser		
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Thr Tyr Lys Ile Met Arg Arg Ile Glu Gly Glu Glu Leu Ala Leu Asp		
370	375	380
Ala Ser Glu Leu Val Ile Thr Ser Thr Arg Gln Glu Ile Asp Glu Gln		
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Trp Gly Leu Tyr Asp Gly Phe Asp Val Lys Leu Glu Lys Val Leu Arg		
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Ala Arg Ala Arg Arg Gly Val Ser Cys His Gly Arg Tyr Met Pro Arg		
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Met Val Val Ile Pro Pro Gly Met Asp Phe Ser Asn Val Val Val His		
435	440	445
Glu Asp Ile Asp Gly Asp Gly Asp Val Lys Asp Asp Ile Val Gly Leu		
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Glu Gly Ala Ser Pro Lys Ser Met Pro Pro Ile Trp Ala Glu Val Met		
465	470	475
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Arg Phe Leu Thr Asn Pro His Lys Pro Met Ile Leu Ala Leu Ser Arg		
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Pro Asp Pro Lys Lys Asn Ile Thr Thr Leu Val Lys Ala Phe Gly Glu		
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Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu Thr Leu Ile Met Gly Asn		
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Arg Asp Asp Ile Asp Asp Met Ser Ala Gly Asn Ala Ser Val Leu Thr		
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Thr Val Leu Lys Leu Ile Asp Lys Tyr Asp Leu Tyr Gly Ser Val Ala		
545	550	555
		560
Phe Pro Lys His His Asn Gln Ala Asp Val Pro Glu Ile Tyr Arg Leu		
565	570	575
Ala Ala Lys Met Lys Gly Val Phe Ile Asn Pro Ala Leu Val Glu Pro		

580	585	590
Phe Gly Leu Thr Leu Ile Glu Ala Ala Ala His Gly Leu Pro Ile Val		
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Ala Thr Lys Asn Gly Gly Pro Val Asp Ile Thr Asn Ala Leu Asn Asn		
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Gly Leu Leu Val Asp Pro His Asp Gln Asn Ala Ile Ala Asp Ala Leu		
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Leu Lys Leu Val Ala Asp Lys Asn Leu Trp Gln Glu Cys Arg Arg Asn		
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Gly Leu Arg Asn Ile His Leu Tyr Ser Trp Pro Glu His Cys Arg Thr		
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Tyr Leu Thr Arg Val Ala Gly Cys Arg Leu Arg Asn Pro Arg Trp Leu		
675	680	685
Lys Asp Thr Pro Ala Asp Ala Gly Ala Asp Glu Glu Glu Phe Leu Glu		
690	695	700
Asp Ser Met Asp Ala Gln Asp Leu Ser Leu Arg Leu Ser Ile Asp Gly		
705	710	715
Glu Lys Ser Ser Leu Asn Thr Asn Asp Pro Leu Trp Phe Asp Pro Gln		
725	730	735
Asp Gln Val Gln Lys Ile Met Asn Asn Ile Lys Gln Ser Ser Ala Leu		
740	745	750
Pro Pro Ser Met Ser Ser Val Ala Ala Glu Gly Thr Gly Ser Thr Met		
755	760	765
Asn Lys Tyr Pro Leu Leu Arg Arg Arg Arg Arg Leu Phe Val Ile Ala		
770	775	780
Val Asp Cys Tyr Gln Asp Asp Gly Arg Ala Ser Lys Lys Met Leu Gln		
785	790	795
Val Ile Gln Glu Val Phe Arg Ala Val Arg Ser Asp Ser Gln Met Phe		
805	810	815
Lys Ile Ser Gly Phe Thr Leu Ser Thr Ala Met Pro Leu Ser Glu Thr		
820	825	830
Leu Gln Leu Leu Gln Leu Gly Lys Ile Pro Ala Thr Asp Phe Asp Ala		

835	840	845
Leu Ile Cys Gly Ser Gly Ser Glu Val Tyr Tyr Pro Gly Thr Ala Asn		
850	855	860
Cys Met Asp Ala Glu Gly Lys Leu Arg Pro Asp Gln Asp Tyr Leu Met		
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		880
His Ile Ser His Arg Trp Ser His Asp Gly Ala Arg Gln Thr Ile Ala		
	885	890
		895
Lys Leu Met Gly Ala Gln Asp Gly Ser Gly Asp Ala Val Glu Gln Asp		
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Val Ala Ser Ser Asn Ala His Cys Val Ala Phe Leu Ile Lys Asp Pro		
915	920	925
Gln Lys Val Lys Thr Val Asp Glu Met Arg Glu Arg Leu Arg Met Arg		
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Gly Leu Arg Cys His Ile Met Tyr Cys Arg Asn Ser Thr Arg Leu Gln		
945	950	955
		960
Val Val Pro Leu Leu Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Ser		
	965	970
		975
Val Arg Trp Gly Val Ser Val Gly Asn Met Tyr Leu Ile Thr Gly Glu		
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His Gly Asp Thr Asp Leu Glu Glu Met Leu Ser Gly Leu His Lys Thr		
995	1000	1005
Val Ile Val Arg Gly Val Thr Glu Lys Gly Ser Glu Ala Leu Val Arg		
1010	1015	1020
Ser Pro Gly Ser Tyr Lys Arg Asp Asp Val Val Pro Ser Glu Thr Pro		
1025	1030	1035
		1040
Leu Ala Ala Tyr Thr Thr Gly Glu Leu Lys Ala Asp Glu Ile Met Arg		
1045	1050	1055
Ala Leu Lys Gln Val Ser Lys Thr Ser Ser Gly Met		
1060	1065	

&lt;210&gt; 9

&lt;211&gt; 1084

&lt;212&gt; PRT

<213> *Oryza sativa*

&lt;400&gt; 9

Met Ala Gly Asn Glu Trp Ile Asn Gly Tyr Leu Glu Ala Ile Leu Asp  
 1 5 10 15

Ser Gly Gly Ala Ala Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly  
 20 25 30

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Val Asp Pro  
 35 40 45

Ser Ser Pro Thr Thr Gly Thr Thr Ser Pro Arg Gly Pro His Met Asn  
 50 55 60

Phe Asn Pro Thr His Tyr Phe Val Glu Glu Val Val Lys Gly Val Asp  
 65 70 75 80

Glu Ser Asp Leu His Arg Thr Trp Ile Lys Val Val Ala Thr Arg Asn  
 85 90 95

Ala Arg Glu Arg Ser Thr Arg Leu Glu Asn Met Cys Trp Arg Ile Trp  
 100 105 110

His Leu Ala Arg Lys Lys Lys Gln Leu Glu Leu Glu Gly Ile Leu Arg  
 115 120 125

Ile Ser Ala Arg Arg Lys Glu Gln Glu Gln Val Arg Arg Glu Thr Ser  
 130 135 140

Glu Asp Leu Ala Glu Asp Leu Phe Glu Gly Glu Lys Ala Asp Thr Val  
 145 150 155 160

Gly Glu Leu Ala Gln Gln Asp Thr Pro Met Lys Lys Lys Phe Gln Arg  
 165 170 175

Asn Phe Ser Glu Leu Thr Val Ser Trp Ser Asp Glu Asn Lys Glu Lys  
 180 185 190

Lys Leu Tyr Ile Val Leu Ile Ser Leu His Gly Leu Val Arg Gly Asp  
 195 200 205

Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys Tyr  
 210 215 220

Val Val Glu Leu Ala Arg Ala Leu Ala Met Met Pro Gly Val Tyr Arg  
 225 230 235 240

Val	Asp	Leu	Phe	Thr	Arg	Gln	Val	Ser	Ser	Pro	Glu	Val	Asp	Trp	Ser	245	250	255
Tyr	Gly	Glu	Pro	Thr	Glu	Met	Leu	Thr	Ser	Gly	Ser	Thr	Asp	Gly	Glu	260	265	270
Gly	Ser	Gly	Glu	Ser	Ala	Gly	Ala	Tyr	Ile	Val	Arg	Ile	Pro	Cys	Gly	275	280	285
Pro	Arg	Asp	Lys	Tyr	Leu	Arg	Lys	Glu	Ala	Leu	Trp	Pro	Tyr	Leu	Gln	290	295	300
Glu	Phe	Val	Asp	Gly	Ala	Leu	Ala	His	Ile	Leu	Asn	Met	Ser	Lys	Ala	305	310	315
Leu	Gly	Glu	Gln	Val	Ser	Asn	Gly	Lys	Leu	Val	Leu	Pro	Tyr	Val	Ile	325	330	335
His	Gly	His	Tyr	Ala	Asp	Ala	Gly	Asp	Val	Ala	Ala	Leu	Leu	Ser	Gly	340	345	350
Ala	Leu	Asn	Val	Pro	Met	Val	Leu	Thr	Gly	His	Ser	Leu	Gly	Arg	Asn	355	360	365
Lys	Leu	Glu	Gln	Ile	Met	Lys	Gln	Gly	Arg	Met	Ser	Lys	Glu	Glu	Met	370	375	380
Asp	Ser	Thr	Tyr	Lys	Ile	Met	Arg	Arg	Ile	Glu	Gly	Glu	Glu	Leu	Ala	385	390	395
Leu	Asp	Ala	Ala	Glu	Leu	Val	Ile	Thr	Ser	Thr	Arg	Gln	Glu	Ile	Asp	405	410	415
Glu	Gln	Trp	Gly	Leu	Tyr	Asp	Gly	Phe	Asp	Val	Lys	Leu	Glu	Lys	Val	420	425	430
Leu	Arg	Ala	Arg	Ala	Arg	Arg	Gly	Val	Ser	Cys	His	Gly	Arg	Phe	Met	435	440	445
Pro	Arg	Met	Val	Val	Ile	Pro	Pro	Gly	Met	Asp	Phe	Ser	Ser	Val	Val	450	455	460
Val	Pro	Glu	Asp	Thr	Ser	Asp	Gly	Asp	Asp	Gly	Lys	Asp	Phe	Glu	Ile	465	470	475
Ala	Ser	Pro	Arg	Ser	Leu	Pro	Pro	Ile	Trp	Ala	Glu	Val	Ser	Arg	Phe	485	490	495

Trp Thr Asn Pro His Lys Pro Met Ile Leu Ala Leu Ser Arg Pro Asp  
 500 505 510

Pro Lys Lys Asn Ile Thr Thr Leu Val Lys Ala Phe Gly Glu Cys Arg  
 515 520 525

Pro Leu Arg Glu Leu Ala Asn Leu Ile Leu Ser Met Gly Thr Arg Asp  
 530 535 540

Asp Ile Asp Gly Met Ser Ala Gly Asn Ala Ser Val Leu Thr Thr Val  
 545 550 555 560

Leu Lys Leu Ile Asp Lys Tyr Asp Leu Tyr Gly Ser Val Ala Phe Pro  
 565 570 575

Lys Tyr His Lys Gln Ser Asp Val Pro Glu Ile Tyr Arg Leu Thr Gly  
 580 585 590

Lys Met Lys Gly Val Phe Ile Asn Pro Ala Leu Val Glu Pro Phe Gly  
 595 600 605

Leu Thr Leu Ile Glu Ala Ala Ala His Gly Leu Pro Ile Val Gly Thr  
 610 615 620

Lys Asn Gly Gly Pro Val Asp Ile Lys Asn Ala Leu Asn Asn Gly Leu  
 625 630 635 640

Leu Val Asp Pro His Asp Gln His Ala Ile Ala Asp Ala Leu Leu Lys  
 645 650 655

Leu Val Ala Asp Lys Asn Leu Trp Gln Glu Cys Arg Lys Asn Gly Leu  
 660 665 670

Arg Asn Ile Gln Leu Tyr Ser Trp Pro Glu His Cys Arg Thr Tyr Leu  
 675 680 685

Thr Arg Ile Ala Gly Cys Arg Ile Arg Asn Pro Arg Trp Leu Met Asp  
 690 695 700

Thr Pro Ala Asp Ala Ala Ala Glu Glu Glu Glu Ala Leu Glu Asp Ser  
 705 710 715 720

Leu Met Asp Val Gln Asp Leu Ser Leu Arg Leu Ser Ile Asp Gly Glu  
 725 730 735

Arg Gly Ser Ser Met Asn Asp Ala Pro Ser Ser Asp Pro Gln Asp Ser  
 740 745 750

Val Gln Arg Ile Met Asn Lys Ile Lys Arg Ser Ser Pro Ala Glu Thr  
 755 760 765  
 Asp Gly Ala Lys Ile Pro Ala Glu Ala Ala Ala Thr Ala Thr Ser Gly  
 770 775 780  
 Ala Met Asn Lys Tyr Pro Leu Leu Arg Arg Arg Arg Arg Leu Phe Val  
 785 790 795 800  
 Ile Ala Val Asp Cys Tyr Gly Asp Asp Gly Ser Ala Ser Lys Arg Met  
 805 810 815  
 Leu Gln Val Ile Gln Glu Val Phe Arg Ala Val Arg Ser Asp Ser Gln  
 820 825 830  
 Met Ser Arg Ile Ser Gly Phe Ala Leu Ser Thr Xaa Met Pro Leu Pro  
 835 840 845  
 Glu Thr Leu Lys Leu Leu Gln Leu Gly Lys Ile Pro Pro Thr Asp Phe  
 850 855 860  
 Asp Ala Leu Ile Cys Gly Ser Gly Ser Glu Val Tyr Tyr Pro Ser Thr  
 865 870 875 880  
 Ala Gln Cys Val Asp Ala Gly Gly Arg Leu Arg Pro Asp Gln Asp Tyr  
 885 890 895  
 Leu Leu His Ile Asn His Arg Trp Ser His Asp Gly Ala Lys Gln Thr  
 900 905 910  
 Ile Ala Lys Leu Ala His Asp Gly Ser Gly Thr Asn Val Glu Pro Asp  
 915 920 925  
 Val Glu Ser Cys Asn Pro His Cys Val Ser Phe Phe Ile Lys Asp Pro  
 930 935 940  
 Asn Lys Val Arg Thr Met Asp Glu Met Arg Glu Arg Val Arg Met Arg  
 945 950 955 960  
 Gly Leu Arg Cys His Leu Met Tyr Cys Arg Asn Ala Thr Arg Leu Gln  
 965 970 975  
 Val Val Pro Leu Leu Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Phe  
 980 985 990  
 Val Arg Trp Gly Leu Ser Val Gly Asn Met Tyr Leu Ile Val Gly Glu  
 995 1000 1005

His Gly Asp Thr Asp His Glu Glu Met Leu Ser Gly Leu His Lys Thr  
 1010 1015 1020

Val Ile Ile Arg Gly Val Thr Glu Lys Gly Ser Glu Gln Leu Val Arg  
 1025 1030 1035 1040

Ser Ser Gly Ser Tyr Gln Arg Glu Asp Val Val Pro Ser Glu Ser Pro  
 1045 1050 1055

Leu Ile Ala Phe Thr Lys Gly Asp Leu Lys Ala Asp Glu Ile Met Arg  
 1060 1065 1070

Ala Leu Lys Glu Val Thr Lys Ala Ala Ser Gly Met  
 1075 1080

<210> 10

<211> 1049

<212> PRT

<213> *Oryza sativa*

<400> 10

Met Ala Gly Asn Glu Trp Ile Asn Gly Tyr Leu Glu Ala Ile Leu Asp  
 1 5 10 15

Ser Gly Gly Ala Ala Gly Gly Gly Gly Gly Gly Gly Gly Val Asp Pro  
 20 25 30

Arg Ser Pro Ala Ala Gly Ala Ala Ser Pro Arg Gly Pro His Met Asn  
 35 40 45

Phe Asn Pro Thr His Tyr Phe Val Glu Glu Val Val Lys Gly Val Asp  
 50 55 60

Glu Ser Asp Leu His Arg Thr Trp Ile Lys Val Val Ala Thr Arg Asn  
 65 70 75 80

Ala Arg Glu Arg Ser Thr Arg Leu Glu Asn Met Cys Trp Arg Ile Trp  
 85 90 95

His Leu Ala Arg Lys Lys Lys Gln Leu Glu Leu Glu Gly Ile Leu Arg  
 100 105 110

Ile Ser Ala Arg Arg Lys Glu Gln Glu Gln Val Arg Arg Glu Thr Ser  
 115 120 125

Glu Asp Leu Ala Glu Asp Leu Phe Glu Gly Glu Lys Ala Asp Thr Val  
 130 135 140



Gly Glu Leu Ala Gln Gln Asp Thr Pro Met Lys Lys Lys Phe Gln Arg  
 145 150 155 160  
 Asn Phe Ser Glu Leu Thr Val Ser Trp Ser Asp Glu Asn Lys Glu Lys  
 165 170 175  
 Lys Leu Tyr Ile Val Leu Ile Ser Leu His Gly Leu Val Ser Gly Asp  
 180 185 190  
 Asn Met Glu Leu Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys Tyr  
 195 200 205  
 Val Val Glu Leu Ala Arg Ala Leu Ala Met Met Pro Gly Val Tyr Arg  
 210 215 220  
 Val Asp Leu Phe Thr Arg Gln Val Ser Ser Pro Glu Val Asp Trp Ser  
 225 230 235 240  
 Tyr Gly Glu Pro Thr Glu Met Leu Thr Pro Val Pro Leu Thr Glu Arg  
 245 250 255  
 Glu Ala Val Arg Val Leu Val Arg Thr Leu Cys Ala Phe Arg Ala Val  
 260 265 270  
 Gln Gly Thr Ser Thr Ser Val Lys Ser Pro Val Ala Leu Pro Pro Arg  
 275 280 285  
 Val Cys Arg Arg Ser Ser Arg Ala Tyr Leu Asn Met Ser Lys Ala Leu  
 290 295 300  
 Gly Glu Gln Val Ser Asn Gly Lys Leu Val Leu Pro Tyr Val Ile His  
 305 310 315 320  
 Gly His Tyr Ala Asp Ala Gly Asp Val Ala Ala Leu Leu Ser Gly Ala  
 325 330 335  
 Leu Asn Val Pro Met Val Leu Thr Gly His Ser Leu Gly Arg Asn Lys  
 340 345 350  
 Leu Glu Gln Ile Met Lys Gln Gly Arg Met Ser Lys Glu Glu Ile Asp  
 355 360 365  
 Ser Thr Tyr Lys Ile Met Arg Arg Ile Glu Gly Glu Glu Leu Ala Leu  
 370 375 380  
 Asp Ala Thr Glu Pro Val Ile Thr Ser Thr Arg Gln Glu Asn Asp Glu  
 385 390 395 400

Gln Trp Gly Leu Tyr Asp Gly Phe Asp Val Lys Leu Glu Lys Val Leu  
 405 410 415  
 Arg Ala Arg Ala Arg Arg Gly Val Ser Cys His Gly Arg Phe Met Pro  
 420 425 430  
 Arg Met Val Val Ile Pro Pro Gly Met Asp Phe Ser Ser Val Val Val  
 435 440 445  
 Pro Glu Asp Thr Ser Asp Gly Asp Asp Gly Lys Asp Phe Glu Ile Ala  
 450 455 460  
 Ser Pro Arg Ser Leu Pro Pro Ile Trp Ala Glu Val Met Arg Phe Leu  
 465 470 475 480  
 Thr Asn Pro His Lys Pro Met Ile Leu Ala Leu Ser Arg Pro Asp Pro  
 485 490 495  
 Lys Lys Asn Ile Thr Thr Leu Val Lys Ala Phe Gly Glu Cys Arg Pro  
 500 505 510  
 Leu Arg Glu Leu Ala Asn Leu Ile Leu Ile Met Gly Asn Arg Asp Asp  
 515 520 525  
 Ile Asp Glu Met Ser Ala Gly Asn Ala Ser Val Leu Thr Thr Val Leu  
 530 535 540  
 Lys Leu Ile Asp Lys Tyr Asp Leu Tyr Gly Ser Val Ala Phe Pro Lys  
 545 550 555 560  
 His His Lys Gln Ser Asp Val Pro Glu Ile Tyr Arg Leu Thr Gly Lys  
 565 570 575  
 Met Lys Gly Val Phe Ile Asn Pro Ala Leu Val Glu Pro Phe Gly Leu  
 580 585 590  
 Thr Leu Ile Glu Ala Ala Ala His Gly Leu Pro Ile Val Ala Thr Lys  
 595 600 605  
 Asn Gly Gly Pro Val Asp Ile Lys Asn Ala Leu Asn Asn Gly Leu Leu  
 610 615 620  
 Val Asp Pro His Asp Gln His Ala Ile Ala Asp Ala Leu Leu Lys Leu  
 625 630 635 640  
 Val Ala Asp Lys Asn Leu Trp Gln Glu Cys Arg Lys Asn Gly Leu Arg  
 645 650 655

Asn Ile Gln Leu Tyr Ser Trp Pro Glu His Cys Arg Thr Tyr Leu Thr  
 660 665 670  
 Arg Ile Ala Gly Cys Arg Ile Arg Asn Pro Arg Trp Leu Met Asp Thr  
 675 680 685  
 Pro Ala Asp Ala Ala Ala Glu Glu Glu Glu Ala Leu Glu Asp Ser Leu  
 690 695 700  
 Met Asp Val Gln Asp Leu Ser Leu His Leu Ser Ile Asp Gly Glu Arg  
 705 710 715 720  
 Gly Ser Ser Met Asn Asp Ala Pro Ser Ser Asp Pro Gln Asp Ser Val  
 725 730 735  
 Gln Arg Ile Met Asn Lys Ile Lys Arg Ser Ser Pro Ala Asp Thr Asp  
 740 745 750  
 Gly Ala Lys Ile Arg Gln Ala Ala Ala Thr Ala Thr Ser Gly Ala Met  
 755 760 765  
 Asn Lys Tyr Pro Leu Leu Arg Arg Arg Arg Arg Leu Phe Val Ile Ala  
 770 775 780  
 Val Asp Cys Tyr Gly Asp Asp Gly Ser Ala Ser Lys Arg Met Leu Gln  
 785 790 795 800  
 Val Ile Gln Glu Val Phe Arg Ala Val Arg Ser Asp Ser Gln Met Ser  
 805 810 815  
 Arg Ile Ser Gly Phe Ala Leu Ser Thr Ala Met Pro Leu Pro Glu Thr  
 820 825 830  
 Leu Lys Leu Leu Gln Leu Gly Lys Ile Pro Pro Thr Asp Phe Asp Ala  
 835 840 845  
 Leu Ile Cys Gly Ser Gly Ser Glu Val Tyr Tyr Pro Gly Thr Ala Gln  
 850 855 860  
 Cys Val Asp Ala Gly Gly Leu Arg Pro Asp Gln Asp Tyr Leu Leu His  
 865 870 875 880  
 Ile Asn His Arg Trp Ser His Asp Gly Ala Lys Gln Thr Ile Ala Asn  
 885 890 895  
 Val Ala His Asp Gly Ser Gly Thr Asn Val Glu Pro Asp Val Glu Ser  
 900 905 910

Cys Asn Pro His Cys Val Ser Phe Phe Ile Lys Asp Pro Asn Lys Val  
           915                          920                          925  
 Arg Thr Ala Asp Glu Met Arg Glu Arg Met Arg Met Arg Gly Leu Arg  
           930                          935                          940  
 Cys His Leu Met Tyr Cys Arg Asn Ala Thr Arg Leu Gln Val Val Pro  
 945                          950                          955                          960  
 Leu Leu Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Phe Val Arg Trp  
                           965                          970                          975  
 Gly Leu Ser Val Gly Asn Met Tyr Leu Ile Val Gly Glu His Gly Asp  
                           980                          985                          990  
 Thr Asp His Glu Glu Met Leu Ser Gly Leu His Lys Thr Val Ile Ile  
           995                          1000                          1005  
 Arg Gly Val Thr Glu Lys Gly Ser Glu Gln Leu Val Arg Ser Ser Gly  
           1010                          1015                          1020  
 Ser Tyr Gln Arg Glu Asp Val Phe Pro Ser Glu Ser Pro Leu Ile Ala  
 1025                          1030                          1035                          1040  
 Phe Thr Lys Gly Asp Leu Lys Ala Asp  
                           1045

<210> 11  
 <211> 1083  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 11  
 Met Ala Arg Asn Asp Trp Ile Asn Ser Tyr Leu Glu Ala Ile Leu Asp  
   1                          5                          10                          15  
 Val Gly Thr Ser Lys Lys Lys Arg Phe Glu Ser Asn Ser Lys Ile Val  
           20                          25                          30  
 Gln Lys Leu Gly Asp Ile Asn Ser Lys Asp His Gln Glu Lys Val Phe  
           35                          40                          45  
 Gly Asp Met Asn Gly Lys Asp His Gln Glu Lys Val Phe Ser Pro Ile  
           50                          55                          60  
 Lys Tyr Phe Val Glu Glu Val Val Asn Ser Phe Asp Glu Ser Asp Leu

65		70		75		80									
Tyr	Lys	Thr	Trp	Ile	Lys	Val	Ile	Ala	Thr	Arg	Asn	Thr	Arg	Glu	Arg
				85					90					95	
Ser	Asn	Arg	Leu	Glu	Asn	Ile	Cys	Trp	Arg	Ile	Trp	His	Leu	Ala	Arg
			100					105					110		
Lys	Lys	Lys	Gln	Ile	Val	Trp	Asp	Asp	Gly	Val	Arg	Leu	Ser	Lys	Arg
			115				120					125			
Arg	Ile	Glu	Arg	Glu	Gln	Gly	Arg	Asn	Asp	Ala	Glu	Glu	Asp	Leu	Leu
			130			135					140				
Ser	Glu	Leu	Ser	Glu	Gly	Glu	Lys	Asp	Lys	Asn	Asp	Gly	Glu	Lys	Glu
145					150					155					160
Lys	Ser	Glu	Val	Val	Thr	Thr	Leu	Glu	Pro	Pro	Arg	Asp	His	Met	Pro
				165					170					175	
Arg	Ile	Arg	Ser	Glu	Met	Gln	Ile	Trp	Ser	Glu	Asp	Asp	Lys	Ser	Ser
			180					185					190		
Arg	Asn	Leu	Tyr	Ile	Val	Leu	Ile	Arg	Gln	Val	Glu	Ile	Gly	Phe	Ser
			195				200					205			
Asp	Leu	Phe	Val	Val	Phe	Asn	Met	Leu	Val	Gly	Leu	Thr	Trp	Cys	Leu
			210				215				220				
Tyr	Leu	Val	Pro	Cys	Phe	Thr	Asn	Cys	Ser	Met	His	Gly	Leu	Val	Arg
225					230					235				240	
Gly	Glu	Asn	Met	Glu	Leu	Gly	Arg	Asp	Ser	Asp	Thr	Gly	Gly	Gln	Val
				245					250					255	
Lys	Tyr	Val	Val	Glu	Leu	Ala	Arg	Ala	Leu	Ala	Asn	Thr	Glu	Gly	Val
			260					265					270		
His	Arg	Val	Asp	Leu	Leu	Thr	Arg	Gln	Ile	Ser	Ser	Pro	Glu	Val	Asp
			275				280					285			
Tyr	Ser	Tyr	Gly	Glu	Pro	Val	Glu	Met	Leu	Ser	Cys	Pro	Pro	Glu	Gly
			290				295				300				
Ser	Asp	Ser	Cys	Gly	Ser	Tyr	Ile	Ile	Arg	Ile	Pro	Cys	Gly	Ser	Arg
305					310					315					320
Asp	Lys	Tyr	Ile	Pro	Lys	Glu	Ser	Leu	Trp	Pro	His	Ile	Pro	Glu	Phe

	325		330		335
Val Asp Gly Ala Leu Asn His Ile Val Ser Ile Ala Arg Ser Leu Gly					
	340		345		350
Glu Gln Val Asn Gly Gly Lys Pro Ile Trp Pro Tyr Val Ile His Gly					
	355		360		365
His Tyr Ala Asp Ala Gly Glu Val Ala Ala His Leu Ala Gly Ala Leu					
	370		375		380
Asn Val Pro Met Val Leu Thr Gly His Ser Leu Gly Arg Asn Lys Phe					
	385		390		395
Glu Gln Leu Leu Gln Gln Gly Arg Ile Thr Arg Glu Asp Ile Asp Arg					
		405		410	415
Thr Tyr Lys Ile Met Arg Arg Ile Glu Ala Glu Glu Gln Ser Leu Asp					
	420		425		430
Ala Ala Glu Met Val Val Thr Ser Thr Arg Gln Glu Ile Asp Ala Gln					
	435		440		445
Trp Gly Leu Tyr Asp Gly Phe Asp Ile Lys Leu Glu Arg Lys Leu Arg					
	450		455		460
Val Arg Arg Arg Arg Gly Val Ser Cys Leu Gly Arg Tyr Met Pro Arg					
	465		470		475
Met Val Val Ile Pro Pro Gly Met Asp Phe Ser Tyr Val Leu Thr Gln					
		485		490	495
Asp Ser Gln Glu Pro Asp Gly Asp Leu Lys Ser Leu Ile Gly Pro Asp					
	500		505		510
Arg Asn Gln Ile Lys Lys Pro Val Pro Pro Ile Trp Ser Glu Ile Met					
	515		520		525
Arg Phe Phe Ser Asn Pro His Lys Pro Thr Ile Leu Ala Leu Ser Arg					
	530		535		540
Pro Asp His Lys Lys Asn Val Thr Thr Leu Val Lys Ala Phe Gly Glu					
	545		550		555
Cys Gln Pro Leu Arg Glu Leu Ala Asn Leu Val Leu Ile Leu Gly Asn					
		565		570	575
Arg Asp Asp Ile Glu Glu Met Pro Asn Ser Ser Ser Val Val Leu Met					

47

835	840	845
Val Leu Ala Ser Gly Ser Ser Leu Gln Glu Val Val Asp Ile Thr Gln		
850	855	860
Lys Asn Leu Ile Asn Leu Glu Asp Phe Asp Ala Ile Val Cys Asn Ser		
865	870	875
Gly Ser Glu Ile Tyr Tyr Pro Trp Arg Asp Met Met Val Asp Ala Asp		
	885	890
Tyr Glu Thr His Val Glu Tyr Lys Trp Pro Gly Glu Ser Ile Arg Ser		
	900	905
Val Ile Leu Arg Leu Ile Cys Thr Glu Pro Ala Ala Glu Asp Asp Ile		
	915	920
Thr Glu Tyr Ala Ser Ser Cys Ser Thr Arg Cys Tyr Ala Ile Ser Val		
	930	935
Lys Gln Gly Val Lys Thr Arg Arg Val Asp Asp Leu Arg Gln Arg Leu		
945	950	955
Arg Met Arg Gly Leu Arg Cys Asn Ile Val Tyr Thr His Ala Ala Thr		
	965	970
Arg Leu Asn Val Ile Pro Leu Cys Ala Ser Arg Ile Gln Ala Leu Arg		
	980	985
Tyr Leu Ser Ile Arg Trp Gly Ile Asp Met Ser Lys Thr Val Phe Phe		
	995	1000
Leu Gly Glu Lys Gly Asp Thr Asp Tyr Glu Asp Leu Leu Gly Gly Leu		
1010	1015	1020
His Lys Thr Ile Ile Leu Lys Gly Val Val Gly Ser Asp Ser Glu Lys		
1025	1030	1035
Leu Leu Arg Ser Glu Glu Asn Phe Lys Arg Glu Asp Ala Val Pro Gln		
	1045	1050
Glu Ser Pro Asn Ile Ser Tyr Val Lys Glu Asn Gly Gly Ser Gln Glu		
	1060	1065
Ile Met Ser Thr Leu Glu Ala Tyr Gly Ile Lys		
1075	1080	



&lt;210&gt; 12

&lt;211&gt; 963

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 12

Met Ala Gly Asn Asp Asn Trp Ile Asn Ser Tyr Leu Asp Gly Ile Leu  
 1 5 10 15

Asp Ala Gly Lys Ala Ala Ile Gly Gly Asn Arg Pro Ser Leu Leu Leu  
 20 25 30

Arg Glu Arg Gly His Phe Ser Pro Ala Arg Tyr Phe Val Glu Glu Val  
 35 40 45

Ile Thr Gly Tyr Asp Glu Thr Asp Leu Tyr Lys Thr Trp Leu Arg Ala  
 50 55 60

Asn Ala Met Arg Ser Arg Arg Glu Glu His Ala Leu Glu Asn Met Thr  
 65 70 75 80

Trp Arg Ile Trp Asn Leu Ala Arg Lys Lys Lys Glu Phe Glu Lys Glu  
 85 90 95

Glu Ala Cys Arg Leu Ser Lys Arg Gln Pro Glu Thr Glu Lys Thr Arg  
 100 105 110

Ala Asp Ala Thr Ala Asp Met Ser Glu Asp Leu Phe Glu Gly Glu Lys  
 115 120 125

Gly Glu Asp Ala Gly Asp Pro Ser Val Ala Tyr Gly Asp Ser Thr Thr  
 130 135 140

Gly Ser Ser Pro Lys Thr Ser Ser Ile Asp Lys Leu Tyr Ile Val Leu  
 145 150 155 160

Ile Ser Leu His Gly Leu Val Arg Gly Glu Asn Met Glu Leu Gly Arg  
 165 170 175

Asp Ser Asp Thr Gly Gly Gln Val Lys Tyr Val Val Glu Leu Ala Lys  
 180 185 190

Ala Leu Ser Ser Ser Pro Gly Val Tyr Arg Val Asp Leu Leu Thr Arg  
 195 200 205

Gln Ile Leu Ala Pro Asn Phe Asp Arg Ser Tyr Gly Glu Pro Ala Glu  
 210 215 220

Leu Leu Val Ser Thr Ser Gly Lys Asn Ser Lys Gln Glu Lys Gly Glu  
 225 230 235 240  
 Asn Ser Gly Ala Tyr Ile Ile Arg Ile Pro Phe Gly Pro Lys Asp Lys  
 245 250 255  
 Tyr Leu Ala Lys Glu His Leu Trp Pro Phe Ile Gln Glu Phe Val Asp  
 260 265 270  
 Gly Ala Leu Ser His Ile Val Arg Met Ser Lys Ala Ile Gly Glu Glu  
 275 280 285  
 Thr Gly Arg Gly His Pro Val Trp Pro Ser Val Ile His Gly His Tyr  
 290 295 300  
 Ala Ser Ala Gly Ile Ala Ala Ala Leu Leu Leu Gly Ala Leu Asn Leu  
 305 310 315 320  
 Pro Met Ala Phe Thr Gly His Phe Leu Gly Lys Asp Lys Leu Glu Gly  
 325 330 335  
 Leu Leu Lys Gln Gly Arg Gln Thr Arg Glu Gln Ile Asn Met Thr Tyr  
 340 345 350  
 Lys Ile Met Cys Arg Ile Glu Ala Glu Glu Leu Ser Leu Asp Ala Ser  
 355 360 365  
 Glu Ile Val Ile Ala Ser Thr Arg Gln Glu Ile Glu Glu Gln Trp Asn  
 370 375 380  
 Leu Tyr Asp Gly Phe Glu Val Ile Leu Ala Arg Lys Leu Arg Ala Arg  
 385 390 395 400  
 Val Lys Arg Gly Ala Asn Cys Tyr Gly Arg Phe Met Pro Arg Met Val  
 405 410 415  
 Ile Ile Pro Pro Gly Val Glu Phe Gly His Ile Ile His Asp Phe Asp  
 420 425 430  
 Met Asp Gly Glu Glu Glu Asn Pro Ser Pro Ala Ser Glu Asp Pro Pro  
 435 440 445  
 Ile Trp Ser Gln Ile Met Arg Phe Phe Thr Asn Pro Arg Lys Pro Met  
 450 455 460  
 Ile Leu Ala Val Ala Arg Pro Tyr Pro Glu Lys Asn Ile Thr Thr Leu  
 465 470 475 480

Val Lys Ala Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu  
 485 490 495  
 Thr Leu Ile Met Gly Asn Arg Glu Ala Ile Ser Lys Met His Asn Met  
 500 505 510  
 Ser Ala Ala Val Leu Thr Ser Val Leu Thr Leu Ile Asp Glu Tyr Asp  
 515 520 525  
 Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His Lys His Ser Glu Val  
 530 535 540  
 Pro Asp Ile Tyr Arg Leu Ala Ala Arg Thr Lys Gly Ala Phe Val Asn  
 545 550 555 560  
 Val Ala Tyr Phe Glu Gln Phe Gly Val Thr Leu Ile Glu Ala Ala Met  
 565 570 575  
 Asn Gly Leu Pro Ile Ile Ala Thr Lys Asn Gly Ala Pro Val Glu Ile  
 580 585 590  
 Asn Gln Val Leu Asn Asn Gly Leu Leu Val Asp Pro His Asp Gln Asn  
 595 600 605  
 Ala Ile Ala Asp Ala Leu Tyr Lys Leu Leu Ser Asp Lys Gln Leu Trp  
 610 615 620  
 Ser Arg Cys Arg Glu Asn Gly Leu Thr Asn Ile His Gln Phe Ser Trp  
 625 630 635 640  
 Pro Glu His Cys Lys Asn Tyr Leu Ser Arg Ile Leu Thr Leu Gly Pro  
 645 650 655  
 Arg Ser Pro Ala Ile Gly Asn Arg Glu Glu Arg Ser Asn Thr Pro Ile  
 660 665 670  
 Ser Gly Arg Arg Gln Ile Ile Val Ile Ser Val Asp Ser Val Asn Lys  
 675 680 685  
 Glu Asp Leu Val Arg Ile Ile Arg Asn Ala Ile Glu Val Ile His Thr  
 690 695 700  
 Gln Asn Met Ser Gly Ser Ala Gly Phe Val Leu Ser Thr Ser Leu Thr  
 705 710 715 720  
 Ile Ser Glu Ile His Ser Leu Leu Leu Ser Gly Gly Met Leu Pro Thr  
 725 730 735

Asp Phe Asp Ala Phe Ile Cys Asn Ser Gly Ser Asn Ile Tyr Tyr Pro  
                     740                    745                    750  
 Ser Tyr Ser Gly Glu Thr Pro Asn Asn Ser Lys Ile Thr Phe Ala Leu  
                     755                    760                    765  
 Asp Gln Asn His Gln Ser His Ile Glu Tyr Arg Trp Gly Gly Glu Gly  
                     770                    775                    780  
 Leu Arg Lys Tyr Leu Val Lys Trp Ala Thr Ser Val Val Glu Arg Lys  
                     785                    790                    795                    800  
 Gly Arg Thr Glu Arg Gln Ile Ile Phe Glu Asp Pro Glu His Ser Ser  
                     805                    810                    815  
 Ala Tyr Cys Leu Ala Phe Arg Val Val Asn Pro Asn His Leu Pro Pro  
                     820                    825                    830  
 Leu Lys Glu Leu Arg Lys Leu Met Arg Ile Gln Ser Leu Arg Cys Asn  
                     835                    840                    845  
 Ala Leu Tyr Asn His Ser Ala Thr Arg Leu Ser Val Val Pro Ile His  
                     850                    855                    860  
 Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Cys Ile Arg Trp Gly Ile  
                     865                    870                    875                    880  
 Glu Val Pro Asn Val Ala Val Leu Val Gly Glu Ser Gly Asp Ser Asp  
                     885                    890                    895  
 Tyr Glu Glu Leu Leu Gly Gly Leu His Arg Thr Val Ile Leu Lys Gly  
                     900                    905                    910  
 Glu Phe Asn Thr Pro Ala Asn Arg Ile His Thr Val Arg Arg Tyr Pro  
                     915                    920                    925  
 Leu Gln Asp Val Val Pro Leu Asp Ser Ser Asn Ile Thr Gly Val Glu  
                     930                    935                    940  
 Gly Tyr Thr Thr Asp Asp Leu Lys Ser Ala Leu Gln Gln Met Gly Ile  
                     945                    950                    955                    960  
 Leu Thr Gln

&lt;210&gt; 13

&lt;211&gt; 963

&lt;212&gt; PRT

&lt;213&gt; Saccharum officinarum

&lt;400&gt; 13

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Met Ala Gly Asn Asp Asn Trp Ile Asn Ser Tyr Leu Asp Gly Ile Leu
  1              5              10              15

Asp Ala Gly Lys Ala Ala Ile Gly Gly Asn Arg Pro Ser Leu Leu Leu
      20              25              30

Arg Glu Arg Gly His Phe Ser Pro Ala Arg Tyr Phe Val Glu Glu Val
      35              40              45

Ile Thr Gly Tyr Asp Glu Thr Asp Leu Tyr Lys Thr Trp Leu Arg Ala
      50              55              60

Asn Ala Met Arg Ser Arg Arg Glu Glu His Ala Leu Glu Asn Met Thr
      65              70              75              80

Trp Arg Ile Trp Asn Leu Ala Arg Lys Lys Lys Glu Phe Glu Lys Glu
      85              90              95

Glu Ala Cys Arg Leu Ser Lys Arg Gln Pro Glu Thr Glu Lys Thr Arg
      100             105             110

Ala Asp Ala Thr Ala Asp Met Ser Glu Asp Leu Phe Glu Gly Glu Lys
      115             120             125

Gly Glu Asp Ala Gly Asp Pro Ser Val Ala Tyr Gly Asp Ser Thr Thr
      130             135             140

Gly Ser Ser Pro Lys Thr Ser Ser Ile Asp Lys Leu Tyr Ile Val Leu
      145             150             155             160

Ile Ser Leu His Gly Leu Val Arg Gly Glu Asn Met Glu Leu Gly Arg
      165             170             175

Asp Ser Asp Thr Gly Gly Gln Val Lys Tyr Val Val Glu Leu Ala Lys
      180             185             190

Ala Leu Ser Ser Ser Pro Gly Val Tyr Arg Val Asp Leu Leu Thr Arg
      195             200             205

Gln Ile Leu Ala Pro Asn Phe Asp Arg Ser Tyr Gly Glu Pro Ala Glu
      210             215             220

Leu Leu Val Ser Thr Ser Gly Lys Asn Ser Lys Gln Glu Lys Gly Glu
      225             230             235             240

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Asn Ser Gly Ala Tyr Ile Ile Arg Ile Pro Phe Gly Pro Lys Asp Lys  
 245 250 255  
 Tyr Leu Ala Lys Glu His Leu Trp Pro Phe Ile Gln Glu Phe Val Asp  
 260 265 270  
 Gly Ala Leu Ser His Ile Val Arg Met Ser Lys Ala Ile Gly Glu Glu  
 275 280 285  
 Thr Gly Arg Gly His Pro Val Trp Pro Ser Val Ile His Gly His Tyr  
 290 295 300  
 Ala Ser Ala Gly Ile Ala Ala Ala Leu Leu Leu Gly Ala Leu Asn Leu  
 305 310 315 320  
 Pro Met Ala Phe Thr Gly His Phe Leu Gly Lys Asp Lys Leu Glu Gly  
 325 330 335  
 Leu Leu Lys Gln Gly Arg Gln Thr Arg Glu Gln Ile Asn Met Thr Tyr  
 340 345 350  
 Lys Ile Met Cys Arg Ile Glu Ala Glu Glu Leu Ser Leu Asp Ala Ser  
 355 360 365  
 Glu Ile Val Ile Ala Ser Thr Arg Gln Glu Ile Glu Glu Gln Trp Asn  
 370 375 380  
 Leu Tyr Asp Gly Phe Glu Val Ile Leu Ala Arg Lys Leu Arg Ala Arg  
 385 390 395 400  
 Val Lys Arg Gly Ala Asn Cys Tyr Gly Arg Phe Met Pro Arg Met Val  
 405 410 415  
 Ile Ile Pro Pro Gly Val Glu Phe Gly His Ile Ile His Asp Phe Asp  
 420 425 430  
 Met Asp Gly Glu Glu Glu Asn Pro Ser Pro Ala Ser Glu Asp Pro Pro  
 435 440 445  
 Ile Trp Ser Gln Ile Met Arg Phe Phe Thr Asn Pro Arg Lys Pro Met  
 450 455 460  
 Ile Leu Ala Val Ala Arg Pro Tyr Pro Glu Lys Asn Ile Thr Thr Leu  
 465 470 475 480  
 Val Lys Ala Phe Gly Glu Cys Arg Pro Leu Arg Glu Leu Ala Asn Leu  
 485 490 495

Thr Leu Ile Met Gly Asn Arg Glu Ala Ile Ser Lys Met His Asn Met  
 500 505 510  
 Ser Ala Ala Val Leu Thr Ser Val Leu Thr Leu Ile Asp Glu Tyr Asp  
 515 520 525  
 Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His Lys His Ser Glu Val  
 530 535 540  
 Pro Asp Ile Tyr Arg Leu Ala Ala Arg Thr Lys Gly Ala Phe Val Asn  
 545 550 555 560  
 Val Ala Tyr Phe Glu Gln Phe Gly Val Thr Leu Ile Glu Ala Ala Met  
 565 570 575  
 Asn Gly Leu Pro Ile Ile Ala Thr Lys Asn Gly Ala Pro Val Glu Ile  
 580 585 590  
 Asn Gln Val Leu Asn Asn Gly Leu Leu Val Asp Pro His Asp Gln Asn  
 595 600 605  
 Ala Ile Ala Asp Ala Leu Tyr Lys Leu Leu Ser Asp Lys Gln Leu Trp  
 610 615 620  
 Ser Arg Cys Arg Glu Asn Gly Leu Thr Asn Ile His Gln Phe Ser Trp  
 625 630 635 640  
 Pro Glu His Cys Lys Asn Tyr Leu Ser Arg Ile Leu Thr Leu Gly Pro  
 645 650 655  
 Arg Ser Pro Ala Ile Gly Asn Arg Glu Glu Arg Ser Asn Thr Pro Ile  
 660 665 670  
 Ser Gly Arg Arg Gln Ile Ile Val Ile Ser Val Asp Ser Val Asn Lys  
 675 680 685  
 Glu Asp Leu Val Arg Ile Ile Arg Asn Ala Ile Glu Val Ile His Thr  
 690 695 700  
 Gln Asn Met Ser Gly Ser Ala Gly Phe Val Leu Ser Thr Ser Leu Thr  
 705 710 715 720  
 Ile Ser Glu Ile His Ser Leu Leu Leu Ser Gly Gly Met Leu Pro Thr  
 725 730 735  
 Asp Phe Asp Ala Phe Ile Cys Asn Ser Gly Ser Asn Ile Tyr Tyr Pro  
 740 745 750

Ser Tyr Ser Gly Glu Thr Pro Asn Asn Ser Lys Ile Thr Phe Ala Leu  
 755 760 765  
 Asp Gln Asn His Gln Ser His Ile Glu Tyr Arg Trp Gly Gly Glu Gly  
 770 775 780  
 Leu Arg Lys Tyr Leu Val Lys Trp Ala Thr Ser Val Val Glu Arg Lys  
 785 790 795 800  
 Gly Arg Thr Glu Arg Gln Ile Ile Phe Glu Asp Pro Glu His Ser Ser  
 805 810 815  
 Ala Tyr Cys Leu Ala Phe Arg Val Val Asn Pro Asn His Leu Pro Pro  
 820 825 830  
 Leu Lys Glu Leu Arg Lys Leu Met Arg Ile Gln Ser Leu Arg Cys Asn  
 835 840 845  
 Ala Leu Tyr Asn His Ser Ala Thr Arg Leu Ser Val Val Pro Ile His  
 850 855 860  
 Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Cys Ile Arg Trp Gly Ile  
 865 870 875 880  
 Glu Val Pro Asn Val Ala Val Leu Val Gly Glu Ser Gly Asp Ser Asp  
 885 890 895  
 Tyr Glu Glu Leu Leu Gly Gly Leu His Arg Thr Val Ile Leu Lys Gly  
 900 905 910  
 Glu Phe Asn Thr Pro Ala Asn Arg Ile His Thr Val Arg Arg Tyr Pro  
 915 920 925  
 Leu Gln Asp Val Val Pro Leu Asp Ser Ser Asn Ile Thr Gly Val Glu  
 930 935 940  
 Gly Tyr Thr Thr Asp Asp Leu Lys Ser Ala Leu Gln Gln Met Gly Ile  
 945 950 955 960  
 Leu Thr Gln

&lt;210&gt; 14

&lt;211&gt; 720

&lt;212&gt; PRT

&lt;213&gt; Synechocystis sp.



<400> 14  
 Met Ser Tyr Ser Ser Lys Tyr Ile Leu Leu Ile Ser Val His Gly Leu  
 1 5 10 15  
 Ile Arg Gly Glu Asn Leu Glu Leu Gly Arg Asp Ala Asp Thr Gly Gly  
 20 25 30  
 Gln Thr Lys Tyr Val Leu Glu Leu Ala Arg Ala Leu Val Lys Asn Pro  
 35 40 45  
 Gln Val Ala Arg Val Asp Leu Leu Thr Arg Leu Ile Lys Asp Pro Lys  
 50 55 60  
 Val Asp Ala Asp Tyr Ala Gln Pro Arg Glu Leu Ile Gly Asp Arg Ala  
 65 70 75 80  
 Gln Ile Val Arg Ile Glu Cys Gly Pro Glu Glu Tyr Ile Ala Lys Glu  
 85 90 95  
 Met Leu Trp Asp Tyr Leu Asp Asn Phe Ala Asp His Ala Leu Asp Tyr  
 100 105 110  
 Leu Lys Glu Gln Pro Glu Leu Pro Asp Val Ile His Ser His Tyr Ala  
 115 120 125  
 Asp Ala Gly Tyr Val Gly Thr Arg Leu Ser His Gln Leu Gly Ile Pro  
 130 135 140  
 Leu Val His Thr Gly His Ser Leu Gly Arg Ser Lys Arg Thr Arg Leu  
 145 150 155 160  
 Leu Leu Ser Gly Ile Lys Ala Asp Glu Ile Glu Ser Arg Tyr Asn Met  
 165 170 175  
 Ala Arg Arg Ile Asn Ala Glu Glu Glu Thr Leu Gly Ser Ala Ala Arg  
 180 185 190  
 Val Ile Thr Ser Thr His Gln Glu Ile Ala Glu Gln Tyr Ala Gln Tyr  
 195 200 205  
 Asp Tyr Tyr Gln Pro Asp Gln Met Leu Val Ile Pro Pro Gly Thr Asp  
 210 215 220  
 Leu Glu Lys Phe Tyr Pro Pro Lys Gly Asn Glu Trp Glu Thr Pro Ile  
 225 230 235 240  
 Val Gln Glu Leu Gln Arg Phe Leu Arg His Pro Arg Lys Pro Ile Ile

245	250	255
Leu Ala Leu Ser Arg Pro Asp Pro Arg Lys Asn Ile His Lys Leu Ile		
260	265	270
Ala Ala Tyr Gly Gln Ser Pro Gln Leu Gln Ala Gln Ala Asn Leu Val		
275	280	285
Ile Val Ala Gly Asn Arg Asp Asp Ile Thr Asp Leu Asp Gln Gly Pro		
290	295	300
Arg Glu Val Leu Thr Asp Leu Leu Leu Thr Ile Asp Arg Tyr Asp Leu		
305	310	315
Tyr Gly Lys Val Ala Tyr Pro Lys Gln Asn Gln Ala Glu Asp Val Tyr		
325	330	335
Ala Leu Phe Arg Leu Thr Ala Leu Ser Gln Gly Val Phe Ile Asn Pro		
340	345	350
Ala Leu Thr Glu Pro Phe Gly Leu Thr Leu Ile Glu Ala Ala Ala Cys		
355	360	365
Gly Val Pro Ile Val Ala Thr Glu Asp Gly Gly Pro Val Asp Ile Ile		
370	375	380
Lys Asn Cys Gln Asn Gly Tyr Leu Ile Asn Pro Leu Asp Glu Val Asp		
385	390	395
Ile Ala Asp Lys Leu Leu Lys Val Leu Asn Asp Lys Gln Gln Trp Gln		
405	410	415
Phe Leu Ser Glu Ser Gly Leu Glu Gly Val Lys Arg His Tyr Ser Trp		
420	425	430
Pro Ser His Val Glu Ser Tyr Leu Glu Ala Ile Asn Ala Leu Thr Gln		
435	440	445
Gln Thr Ser Val Leu Lys Arg Ser Asp Leu Lys Arg Arg Arg Thr Leu		
450	455	460
Tyr Tyr Asn Gly Ala Leu Val Thr Ser Leu Asp Gln Asn Leu Leu Gly		
465	470	475
Ala Leu Gln Gly Gly Leu Pro Gly Asp Arg Gln Thr Leu Asp Glu Leu		
485	490	495
Leu Glu Val Leu Tyr Gln His Arg Lys Asn Val Gly Phe Cys Ile Ala		

500	505	510
Thr Gly Arg Arg Leu Asp Ser Val Leu Lys Ile Leu Arg Glu Tyr Arg 515	520	525
Ile Pro Gln Pro Asp Met Leu Ile Thr Ser Met Gly Thr Glu Ile Tyr 530	535	540
Ser Ser Pro Asp Leu Ile Pro Asp Gln Ser Trp Arg Asn His Ile Asp 545	550	555 560
Tyr Leu Trp Asn Arg Asn Ala Ile Val Arg Ile Leu Gly Glu Leu Pro 565	570	575
Gly Leu Ala Leu Gln Pro Lys Glu Glu Leu Ser Ala Tyr Lys Ile Ser 580	585	590
Tyr Phe Tyr Asp Ala Ala Ile Ala Pro Asn Leu Glu Glu Ile Arg Gln 595	600	605
Leu Leu His Lys Gly Glu Gln Thr Val Asn Thr Ile Ile Ser Phe Gly 610	615	620
Gln Phe Leu Asp Ile Leu Pro Ile Arg Ala Ser Lys Gly Tyr Ala Val 625	630	635 640
Arg Trp Leu Ser Gln Gln Trp Asn Ile Pro Leu Glu His Val Phe Thr 645	650	655
Ala Gly Gly Ser Gly Ala Asp Glu Asp Met Met Arg Gly Asn Thr Leu 660	665	670
Ser Val Val Val Ala Asn Arg His His Glu Glu Leu Ser Asn Leu Gly 675	680	685
Glu Ile Glu Pro Ile Tyr Phe Ser Glu Lys Arg Tyr Ala Ala Gly Ile 690	695	700
Leu Asp Gly Leu Ala His Tyr Arg Phe Phe Glu Leu Leu Asp Pro Val 705	710	715 720

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/24490

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A01H 5/00, 5/10

US CL :800/314

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/314

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
STN, AGRICOLA, CAPLUS, BIOSIS, EMBASE, USPAT  
search terms: sucrose phosphate synthase, DNA, cDNA, gene, nucleic, plant, transgenic, transform, cotton, gossypium

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,914,446 A (SHEWMAKER) 22 June 1999, see entire patent.	1-10
Y	US 5,665,892 A (VAN ASSCHE et al) 09 September 1997, see entire patent.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

03 NOVEMBER 2000

Date of mailing of the international search report

27 DEC 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

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PARALEGAL SPECIALIST  
CHEMICAL MATRIX

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/24490

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-10

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/24490

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-10, drawn to transgenic cotton plant with increased sucrose phosphate synthase.

Group II, claim(s) 11-23, drawn to method of increasing yield of a cotton plant.

Group III, claim(s) 24-35, drawn to method of increasing quality of cotton fiber in a cotton plant.

Group IV, claim(s) 36-51, drawn to method of regulating the ratio of cellulose to other dry weight components in a plant.

Group V, claim(s) 52-62, drawn to method of increasing tolerance of photosynthetic efficiency to cool night temperatures in a plant.

Group VI, claim(s) 63-69, drawn to method of regulating the thickness of cell walls in a plant.

Group VII, claim(s) 70-74, drawn to method of increasing the harvestable yield of fiber in a fiber containing plant.

Group VIII, claim(s) 75-79, drawn to method of increasing the harvestable yield of seed in a plant.

Group IX, claim(s) 80-82, drawn to method of altering the quality of fiber isolated from a fiber producing plant.

The inventions listed as Groups I, II, III, IV, V, VI, VII, VIII, and IX do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The transgenic cotton plant with increased sucrose phosphate synthase of Group I encompasses plants transformed with many different DNAs encoding many different enzymes or encoding many different antisense RNAs. Therefore, there is no single special technical feature which links the transgenic cotton plant of Group I, with any of the methods of Groups II, III, IV, V, VI, VII, and VIII.

The methods of Groups II, III, IV, V, VI, VII, and VIII are distinct methods differing in starting material and end product. Therefore, the inventions of Groups I, II, III, IV, V, VI, VII, VIII, and IX do not relate to a single inventive concept under PCT Rule 13.1.